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(54) Title: MOLECULES THAT HOME TO A SELECTED ORGAN OR TISSUE IN VIVO AND METHODS OF IDENTIFYING SAME

(57) Abstract

The present invention provides an *in vivo* method for identifying molecules that home to a selected organ or tissue. In addition, the invention provides peptides that home to a selected organ or tissue. For example, the invention provides peptides that selectively home to an organ such as brain or kidney or to a tissue such as a tumor tissue. The invention further provides methods of using an organ homing molecule, for example, to target an agent such as a drug to a selected organ or to identify the target molecule expressed by the selected organ. The invention also provides methods of targeting an organ or tissue containing angiogenic vasculature by contacting the organ or tissue with a molecule that specifically binds an α_v-containing integrin.

MOLECULES THAT HOME TO A SELECTED ORGAN OR TISSUE
IN VIVO AND METHODS OF IDENTIFYING SAME

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to the
10   fields of molecular medicine and drug delivery and, more
specifically, to a method of *in vivo* panning for
identifying a molecule that homes to a specific organ and
to molecules that home to specific organs, including to
normal organs and to tumors.

15   ### BACKGROUND INFORMATION

Although the effect of a particular pathology
often is manifest throughout the body of the afflicted
person, generally, the underlying pathology may affect
only a single organ or tissue. In many cases, drugs are
20   the treatment of choice for a patient suffering a
particular disease. It is rare, however, that a drug
will target only the diseased tissue or organ. More
commonly, drug treatment results in undesirable side
effects due, for example, to generalized toxic effects
25   throughout the patient's body. The nausea, loss of hair
and drop in blood count that occur as a result of
treating of a cancer patient with chemotherapeutic agents
are examples of the undesirable side effects that can
occur due to drug treatment.

30   The undesirable side effects that can occur
when drugs are used to treat a disease most often are due
to the inability of the drug to specifically target the

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diseased organ or tissue. For example, a cancer
chemotherapeutic agent that targets rapidly proliferating
cells would be useful to kill rapidly dividing cancer
cells. However, such an agent also kills normal
5   proliferating hematopoietic and epithelial cells. Thus,
the dose of such a drug that can be administered to a
patient is limited due to its toxic effect on normal
cells.

Efforts have been made to increase the target
10 specificity of various drugs. In some cases, a
particular cell type present in a diseased tissue or
organ may express a unique cell surface marker. In such
a case, an antibody can be raised against the unique cell
surface marker and a drug can be linked to the antibody.
15 Upon administration of the drug/antibody complex to the
patient, the binding of the antibody to the cell surface
marker results in the delivery of a relatively high
concentration of the drug to the diseased tissue or
organ. Similar methods can be used where a particular
20 cell type in the diseased organ expresses a unique cell
surface receptor or a ligand for a particular receptor.
In these cases, the drug can be linked to the specific
ligand or to the receptor, respectively, thus providing a
means to deliver a relatively high concentration of the
25 drug to the diseased organ.

While linking a drug to a molecule that homes
to a particular cell type present in a diseased organ or
tissue provides significant advantages for treatment over
the use of a drug, alone, use of this method is severely
30 limited. In particular, very few cell type specific
antibodies have been described and it can be difficult
and time consuming to attempt to obtain an antibody that
targets an organ in a particular patient suffering a
pathology. Furthermore, few cell type specific surface
35 markers have been described. Even where such markers

have been described, the cells expressing the markers can
be distributed among various tissues or organs, thereby
limiting their usefulness as targets. Thus, it is
important to identify specific target cell markers that
5   are expressed in only one or a few tissues or organs and
to identify molecules that specifically interact with
such markers.

Various cell types can express unique markers
and, therefore, provide potential targets for organ
10 homing molecules. Endothelial cells, for example, which
line the internal surfaces of blood vessels, can have
distinct morphologies and biochemical markers in
different tissues. The blood vessels of the lymphatic
system, for example, express various adhesion proteins
15 that serve to guide lymphocyte homing. In addition,
endothelial cells present in lymph nodes express a cell
surface marker that is a ligand for L-selectin and
endothelial cells in Peyer's patch venules express a
ligand for the $\alpha_4\beta_7$ integrin. These ligands are involved
20 in specific lymphocyte homing to their respective
lymphoid organs. Thus, linking a drug to L-selectin or
to the $\alpha_4\beta_7$ integrin may provide a means for targeting the
drug to diseased lymph nodes or Peyer's patches,
respectively, provided that these molecules do not bind
25 to similar ligands present in a significant number of
other organs.

Although the homing molecules present in the
blood vessels of non-lymphoid tissues have not been
clearly defined, the ability of lymphocytes to return to
30 the organ in which they were first stimulated indicates
that organ-specific endothelial markers exist.
Similarly, the homing or metastasis of particular types
of tumor cells to specific organs provides further
evidence that organ-specific markers exist. However,

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there remains a need to identify other organ-specific
cell markers and the molecules that bind to them.

Methods are now available for producing large
populations of molecules and for screening libraries of
5   molecules to identify those of interest. For example,
phage peptide display libraries can be used to express
large numbers of peptides that can be screened *in vitro*
with a particular target molecule or a cell of interest
in order to identify peptides that specifically bind the
10   target molecule or the cell. Screening of such phage
display libraries has been used, for example, to identify
ligands that specifically bind various antibodies and
cell surface receptors.

Screening of a phage display library generally
15   involves *in vitro* panning of the library using a purified
target molecule. Phage that bind the target molecule can
be recovered, individual phage can be cloned and the
peptide expressed by a cloned phage can be determined.
Such a peptide can be useful for delivery of a drug
20   linked to the peptide to cells expressing the target
molecule.

Unfortunately, very few target molecules that
are expressed by only one or a few cell types have been
identified. Furthermore, even where such a target
25   molecule is known, it is uncertain whether a peptide that
specifically binds the target molecule, as determined
using an *in vitro* panning method, will bind to the target
molecule *in vivo*. As a result, the identification of a
peptide from a phage display library using an *in vitro*
30   panning method essentially represents only a starting
point for determining whether the identified peptide can
be useful for an *in vivo* procedure. Thus, a need exists
to develop *in vivo* methods for screening large numbers of
molecules such as peptides in order to identify those

that can home to one or more selected organs or tissues.
The present invention satisfies this need and provides
related advantages as well.

SUMMARY OF THE INVENTION

5   The present invention provides a method of
identifying a molecule that homes specifically to one or
a few selected organs or tissues by using *in vivo* panning
to screen a library of potential organ homing molecules.
The *in vivo* panning method involves administering a
10   library, which consists of a diverse population of
molecules, to a subject and identifying molecules that
home to one or more selected organs or tissues in the
subject. The invention is exemplified by administration
of a phage peptide display library to a subject and the
15   identification of peptides expressed by phage that home
to brain, to kidney or to a tumor such as a breast cancer
or a melanoma in the subject. *In vivo* panning can be
repeated one or more times until a molecule that homes to
a selected organ or tissue with the desired selectivity
20   is recovered.

          The present invention also provides peptides
that selectively home to a selected organ or tissue. For
example, the invention provides brain homing peptides
having the motif, SRL (serine-arginine-lysine), such as
25   the peptide CLSSRLDAC (SEQ ID NO: 3), or the motif VLR,
such as the peptide WRCVLREGPAGGCAWFNRHRL (SEQ ID
NO: 16). In addition, the invention provides kidney
homing peptides such as the peptide CLPVASC (SEQ ID
NO: 21). Also, the invention provides tumor homing
30   peptides such as the peptide CGRECPRLCQSSC (SEQ ID
NO: 48), which homes to a breast tumor, and peptides
WGTGLC (SEQ ID NO: 52) and CLSGSLSC (SEQ ID NO: 55),
which home to a melanoma. Such organ or tissue homing
molecules are referred to herein, for convenience, as

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"organ homing" molecules. A "tumor homing molecule" is
an example of an organ homing molecule. An organ homing
molecule of the invention is useful, for example, for
targeting a moiety such as a drug to the selected organ
5   or tissue or for identifying the presence of a target
molecule in a sample.

        The invention further provides methods of
identifying a target molecule by detecting selective
binding of the target molecule to an organ homing
10   molecule. For example, a peptide that selectively homes
to a selected organ or tissue can be attached to a solid
matrix for use in affinity chromatography. A sample of
the selected organ or tissue can be obtained and passed
over the affinity matrix under conditions that allow
15   specific binding of the target molecule, which then can
be collected and identified using well known biochemical
methods. The target molecule can be useful, for example,
for raising an antibody specific for the target molecule.

DETAILED DESCRIPTION OF THE INVENTION

20   The present invention provides a method of
identifying a molecule that homes specifically to one or
a few selected organs or tissues, including to a tumor
such as a melanoma or a breast cáncer, by screening a
library using *in vivo* panning (see Pasqualini and
25   Ruoslahti, Nature 380:364-366 (1996), which is
incorporated herein by reference). The identified organ
homing molecules are useful, for example, for targeting a
desired moiety such as a drug, a toxin or a detectable
label, which can be linked to the molecule, to the
30   selected organ or tissue. *In vivo* panning provides a
direct means for identifying molecules that selectively
home to a selected organ or tissue and, therefore,
provides a significant advantage over previous methods,
which require that a molecule identified using an *in*

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vitro screening method subsequently be examined to determine whether it maintains its specificity *in vivo*.

As used herein, the term "library" means a collection of molecules. A library can contain a few or
5   a large number of different molecules, varying from about ten molecules to several billion molecules or more. If desired, a molecule can be linked to a tag, which can be a shared tag or a specific tag that can facilitate recovery or identification of the molecule.

10   As used herein, the term "molecule" is used broadly to mean an organic chemical such as a drug; a peptide, including a variant or modified peptide or peptide-like molecules such as a peptidomimetic or peptoid; or a protein such as an antibody or a growth
15   factor receptor or a fragment thereof such as an Fv, Fd or Fab fragment of an antibody, which contains a binding domain. For convenience, the term "peptide" is used broadly herein to mean peptides, proteins, fragments of proteins and the like. A molecule can be a non-naturally
20   occurring molecule, which does not occur in nature, but is produced as a result of *in vitro* methods, or can be a naturally occurring molecule such as a protein or fragment thereof expressed from a cDNA library.

Methods for preparing libraries containing
25   diverse populations of various types of molecules such as peptides, peptoids and peptidomimetics are well known in the art and commercially available (see, for example, Ecker and Crooke, Biotechnology 13:351-360 (1995), and Blondelle et al., Trends Anal. Chem. 14:83-92 (1995), and
30   the references cited therein, each of which is incorporated herein by reference; see, also, Goodman and Ro, Peptidomimetics for Drug Design, in "Burger's Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M.E. Wolff; John Wiley & Sons 1995), pages 803-861, and Gordon

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et al., J. Med. Chem. 37:1385-1401 (1994), each of which
is incorporated herein by reference). Where a molecule
is a peptide, protein or fragment thereof, the molecule
can be produced *in vitro* directly or can be expressed
5 from a nucleic acid, which is produced *in vitro*. Methods
of synthetic peptide and nucleic acid chemistry are well
known in the art.

A library of molecules also can be produced,
for example, by constructing a cDNA expression library
10 from mRNA collected from a cell, tissue, organ or
organism of interest. Methods for producing such
libraries are well known in the art (see, for example,
Sambrook et al., Molecular Cloning: A laboratory manual
(Cold Spring Harbor Laboratory Press 1989), which is
15 incorporated herein by reference). Preferably, the
peptide encoded by the cDNA is expressed on the surface
of a cell or a virus containing the cDNA. For example,
cDNA can be cloned into a phage vector such as fuse 5
(see Example I), wherein, upon expression, the encoded
20 peptide is expressed as a fusion protein on the surface
of the phage.

In addition, a library of molecules can
comprise a library of nucleic acid molecules. Nucleic
acid molecules that bind, for example, to a cell surface
25 receptor are known in the art (see O'Connell et al.,
Proc. Natl. Acad. Sci., USA 93:5883-5887 (1996); Tuerk
and Gold, Science 249:505-510 (1990); Gold et al., Ann.
Rev. Biochem. 64:763-797 (1995), each of which is
incorporated herein by reference). Thus, a library of
30 nucleic acid molecules can be administered to a subject
having a tumor and tumor homing molecules can be
identified by *in vivo* panning as disclosed herein. If
desired, the nucleic acid molecules can be nucleic acid
analogs that, for example, are less susceptible to attack
35 by nucleases (see, for example, Jelinek et al.,

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Biochemistry 34:11363-11372 (1995); Latham et al., Nucl.
Acids Res. 22:2817-2822 (1994); Tam et al., Nucl. Acids
Res. 22:977-986 (1994); Reed et al., Cancer Res. 59:6565-
6570 (1990), each of which is incorporated herein by
5   reference).

As disclosed herein, *in vivo* panning comprises
administering a library to a subject, collecting a
selected organ or tissue and identifying an organ homing
molecule. An organ homing molecule can be identified
10 using various methods well known in the art. Generally,
the presence of an organ homing molecule in a selected
organ or tissue is identified based on one or more
characteristics common to the molecules present in the
library, then the structure of a particular organ homing
15 molecule is identified. For example, a highly sensitive
detection method such as mass spectrometry, either alone
or in combination with a method such as gas
chromatography, can be used to identify organ homing
molecules in a selected organ or tissue. Thus, where a
20 library comprises diverse molecules based generally on
the structure of an organic molecule, the presence of an
organ homing molecule such as a drug can be identified by
detecting the presence of a parent peak for the
particular molecule.

25   If desired, the selected organ or tissue can be
collected, then processed using a method such as HPLC,
which can provide a fraction enriched in molecules having
a defined range of molecular weights or polar or nonpolar
characteristics or the like. Conditions for HPLC will
30 depend on the chemistry of the particular molecule and
are well known to those skilled in the art. Similarly,
methods for bulk removal of potentially interfering
cellular materials such as DNA, RNA, proteins, lipids or
carbohydrates are well known in the art as are methods
35 for enriching a fraction containing an organic molecule

using, for example, methods of selective extraction. For
example, where a library comprises a population of
diverse organic chemical molecules each linked to a
specific oligonucleotide tag, such that the particular
5   molecule is identified by determining the oligonucleotide
sequence using the polymerase chain reaction (PCR),
genomic DNA can be removed from the sample of the
collected organ in order to reduce the potential for
background PCR reactions. In addition, a library can
10   comprise a population of diverse molecules such as
organic chemical molecules each linked to a common,
shared tag. Based on the presence and properties of the
shared tag, molecules of the library that selectively
home to an organ or tissue can be substantially isolated
15   from a sample of the organ or tissue. These and other
methods can be useful for enriching the sample of the
collected organ for the particular organ homing molecule,
thereby removing potentially contaminating materials from
the collected organ sample and increasing the sensitivity
20   of detecting the molecule.

        Evidence provided herein indicates that a
sufficient number of organ homing molecules selectively
home to a selected organ or tissue during *in vivo* panning
such that the molecules readily can be identified. For
25   example, various independent phage expressing the same
peptide were identified in brain (Table 1), in kidney
(Table 2), and in a tumor formed from implanted breast
carcinoma cells (Table 3) or from implanted melanoma
cells (Table 4). Specifically, almost half of the kidney
30   homing peptides that were sequenced had the amino acid
sequence CLPVASC (SEQ ID NO: 21); two peptides that homed
to brain constituted about 40% of the sequenced brain
homing peptides; one peptide that homed to the breast
tumor constituted about 43% of the sequenced breast tumor
35   homing peptides; and two peptides that homed to the

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melanoma constituted greater than 75% of the sequenced
melanoma homing peptides (see Examples II and III).

Although a substantial fraction of the
identified organ homing molecules have the same
5   structure, the peptide inserts of only a small number of
isolated phage were determined. It should be recognized,
however, that hundreds of thousands to millions of phage
expressing organ homing peptides were recovered following
various *in vivo* pannings for organ homing molecules.
10   These results indicate that specific organ homing
molecules will be present in substantial numbers in an
organ following *in vivo* homing, thereby increasing the
ease with which the molecules can be identified.

Ease of identification of an organ homing
15   molecule, particularly an untagged molecule, depends on
various factors, including the presence of potentially
contaminating background cellular material. Thus, where
the organ homing molecule is an untagged peptide, a
larger number must home to the organ in order to identify
20   the specific peptides against the background of cellular
protein. In contrast, a much smaller number of an
untagged organic chemical homing molecule is identifiable
because such molecules normally are absent from or
present in only small numbers in the body. In such a
25   case, a highly sensitive method such as mass spectrometry
can be used to identify an organ homing molecule. The
skilled artisan will recognize that the method of
identifying a molecule will depend, in part, on the
chemistry of the particular molecule.

30   Where an organ homing molecule is a nucleic
acid or is tagged with a nucleic acid molecule, an assay
such as PCR can be particularly useful for identifying
the presence of the molecule because, in principle, PCR
can detect the presence of a single nucleic acid molecule

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(see, for example, Erlich, PCR Technology: Principles
and Applications for DNA Amplification (Stockton Press
1989), which is incorporated herein by reference).
Preliminary studies have demonstrated that, following
5   intravenous injection of 10 ng of an approximately 6000
base pair plasmid into a mouse and 2 minutes in the
circulation, the plasmid was detectable by PCR in a
sample of lung. These results indicate that nucleic
acids are sufficiently stable when administered into the
10 circulation such that in vivo panning can be used to
identify nucleic acid molecules that selectively home to
a selected organ.

        The molecules of a library can be tagged, which
can facilitate recovery or identification of the
15 molecule. As used herein, the term "tag" means a
physical, chemical or biological moiety such as a plastic
microbead, an oligonucleotide or a bacteriophage,
respectively, that is linked to a molecule of the
library. Methods for tagging a molecule are well known
20 in the art (Hermanson, Bioconjugate Techniques (Academic
Press 1996), which is incorporated herein by reference).

        A tag, which can be a shared tag or a specific
tag, can be useful for identifying the presence or
structure of a molecule of a library. As used herein,
25 the term "shared tag" means a physical, chemical or
biological moiety that is common to each molecule in a
library. Biotin, for example, can be a shared tag that
is linked to each molecule in a library. A shared tag
can be useful to identify the presence of a molecule of
30 the library in a sample and also can be useful to
substantially isolate the molecules from a sample. For
example, where the shared tag is biotin, the biotin-
tagged molecules in a library can be substantially
isolated by binding to streptavidin or their presence can
35 be identified by binding with a labeled streptavidin.

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Where a library is a phage display library, the phage that express the peptides are another example of a shared tag, since each peptide of the library is linked to a phage. In addition, a peptide such as the hemaglutinin antigen can be a shared tag that is linked to each molecule in a library, thereby allowing the use of an antibody specific for the hemaglutinin antigen to substantially isolate molecules of the library from a sample of a selected organ or tissue.

A shared tag also can be a nucleic acid sequence that can be useful to identify the presence of molecules of the library in a sample or to substantially isolate molecules of a library from a sample. For example, each of the molecules of a library can be linked to the same selected nucleotide sequence, which constitutes the shared tag. An affinity column containing a nucleotide sequence that is complementary to the shared tag then can be used to hybridize molecules of the library containing the shared tag, thus substantially isolating the molecules from an organ or tissue sample. A nucleotide sequence complementary to a portion of the shared nucleotide sequence tag also can be used as a PCR primer such that the presence of molecules containing the shared tag can be identified in a sample by PCR.

A tag also can be a specific tag. As used herein, the term "specific tag" means a physical, chemical or biological tag that is linked to a particular molecule in a library and is unique for that particular molecule. A specific tag is particularly useful if it is readily identifiable. A nucleotide sequence that is unique for a particular molecule of a library is an example of a specific tag. For example, the method of synthesizing peptides tagged with a unique nucleotide sequence provides a library of molecules, each containing a specific tag, such that upon determining the nucleotide

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sequence, the identity of the peptide is known (see
Brenner and Lerner, Proc. Natl. Acad. Sci., USA 89:5381-
5383 (1992), which is incorporated herein by reference).
The use of a nucleotide sequence as a specific tag for a
5   peptide or other type of molecule provides a simple means
to identify the presence of the molecule in a sample
because an extremely sensitive method such as PCR can be
used to determine the nucleotide sequence of the specific
tag, thereby identifying the sequence of the molecule
10   linked thereto. Similarly, the nucleic acid sequence
encoding a peptide expressed on a phage is another
example of a specific tag, since sequencing of the
specific tag identifies the amino acid sequence of the
expressed peptide.

15   The presence of a shared tag or a specific tag
can provide a means to identify or recover an organ
homing molecule of the invention following *in vivo*
panning. In addition, the combination of a shared tag
and specific tag can be particularly useful for
20   identifying an organ homing molecule. For example, a
library of peptides can be prepared such that each is
linked to a specific nucleotide sequence tag (see, for
example, Brenner and Lerner, *supra*, 1992), wherein each
specific nucleotide sequence tag has incorporated therein
25   a shared tag such as biotin. Upon homing to an organ,
the particular organ homing peptides can be substantially
isolated from a sample of the organ based on the shared
tag and the specific peptides can be identified, for
example, by PCR of the specific tag (see Erlich, *supra*,
30   1989).

A tag also can serve as a support. As used
herein, the term "support" means a tag having a defined
surface to which a molecule can be attached. In general,
a tag useful as a support is a shared tag. For example,
35   a support can be a biological tag such as a virus or

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virus-like particle such as a bacteriophage ("phage"); a
bacterium such as *E. coli*; or a eukaryotic cell such as a
yeast, insect or mammalian cell; or can be a physical tag
such as a liposome or a microbead, which can be composed
5   of a plastic, agarose, gelatin or other material. If
desired, a shared tag useful as a support can have linked
thereto a specific tag. Thus, the phage display
libraries used in the exemplified methods can be
considered to consist of the phage, which is a shared tag
10 that also is a support, and the nucleic acid sequence
encoding the expressed peptide, the nucleic acid sequence
being a specific tag.

In general, a support should have a diameter
less than about 10 μm to about 50 μm in its shortest
15 dimension, such that the support can pass relatively
unhindered through the capillary beds present in the
subject and not occlude circulation. In addition, a
support can be nontoxic, so that it does not perturb the
normal expression of cell surface molecules or normal
20 physiology of the subject, and biodegradable,
particularly where the subject used for *in vivo* panning
is not sacrificed to collect a selected organ or tissue.

Where a molecule is linked to a support, the
tagged molecule comprises the molecule attached to the
25 surface of the support, such that the part of the
molecule suspected of being able to interact with a
target in a cell in the subject is positioned so as to be
able to participate in the interaction. For example,
where the molecule is suspected of being a β adrenergic
30 agonist, the binding portion of the molecule attached to
a support is positioned so it can interact with a β
adrenergic receptor on a cell in the selected organ or
tissue. Similarly, where the molecule is suspected of
being a ligand for a growth factor receptor, the molecule
35 is positioned on the support so that it can bind the

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receptor. If desired, an appropriate spacer molecule can
be positioned between the molecule and the support such
that the ability of the potential organ homing molecule
to interact with the target molecule is not hindered. A
5   spacer molecule also can contain a reactive group, which
provides a convenient and efficient means of linking a
molecule to a support and, if desired, can contain a tag,
which can facilitate recovery or identification of the
molecule (see Hermanson, *supra*, 1996).

10   As exemplified herein, a peptide suspected of
being able to home to a selected organ or tissue such as
brain was expressed as the N-terminus of a fusion
protein, wherein the C-terminus consisted of a phage coat
protein. Upon expression of the fusion protein, the
15 C-terminal coat protein linked the fusion protein to the
surface of a phage such that the N-terminal peptide was
in a position to interact with a target molecule in an
organ. Thus, a molecule having a shared tag was formed
by the linking of a peptide to a phage, wherein the phage
20 provided a biological support, the peptide molecule is
linked as a fusion protein, the phage-encoded portion of
the fusion protein acts as a spacer molecule, and the
nucleic acid encoding the peptide provided a specific tag
allowing identification of an organ homing peptide.

25   As used herein, the term "*in vivo* panning"
means a method of screening a library by administering
the library to a subject and identifying a molecule that
selectively homes to one or a few selected organs or
tissues. The term "administering to a subject", when
30 used in referring to a library of molecules or a portion
thereof, is used in its broadest sense to mean that the
library is delivered to a selected organ or tissue,
including, where desired, a tumor, such that the
molecules of the library contact the selected organ or
35 tissue.

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A library can be administered to a subject, for example, by injecting the library into the circulation of the subject such that the molecules can pass through the selected organ or tissue; after an appropriate period of

5   time, circulation is terminated by sacrificing the subject or by removing a sample of the organ (see Example I). Alternatively, a cannula can be inserted into a blood vessel in the subject, such that the library is administered by perfusion for an appropriate period of

10   time, after which the library can be removed from the circulation through the cannula or the subject can be sacrificed or the organ can be sampled to terminate circulation. Similarly, a library can be shunted through one or a few organs by cannulation of the appropriate

15   blood vessels in the subject. It is recognized that a library also can be administered to an isolated perfused organ. Such panning in an isolated perfused organ can be useful for identifying molecules that bind to the organ and, if desired, can be used as an initial screening of a

20   library. For example, if a kidney homing molecule is desired, a library can be perfused through an isolated kidney, then the molecules that bound to the perfused kidney can be screened by *in vivo* panning to identify a kidney homing molecule.

25   The *in vivo* panning method is exemplified herein by screening a phage peptide display library in mice and identifying specific peptides that selectively home to brain, kidney or a tumor (see Examples II and III). However, phage libraries that display protein

30   receptor molecules, including, for example, an antibody or an antigen binding fragment of an antibody such an Fv, Fd or Fab fragment; a hormone receptor such as a growth factor receptor; or a cell adhesion receptor such as an integrin or a selectin also can be used to practice the

35   invention. Variants of such molecules can be constructed using well known methods such as random, site-directed or

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codon based mutagenesis (see Huse, U.S. Patent
No. 5,264,563, issued November 23, 1993, which is
incorporated herein by reference). Thus, various types
of phage display libraries can be screened using the
5   disclosed *in vivo* panning method.

Phage display technology provides a means for
expressing a diverse population of random or selectively
randomized peptides. Various methods of phage display
and methods for producing diverse populations of peptides
10   are well known in the art. For example, Ladner et al.
(U.S. Patent No. 5,223,409, issued June 29, 1993, which
is incorporated herein by reference) describe methods for
preparing diverse populations of binding domains on the
surface of a phage. In particular, Ladner et al.
15   describe phage vectors useful for producing a phage
display library, as well as methods for selecting
potential binding domains and producing randomly or
selectively mutated binding domains.

Similarly, Smith and Scott (Meth. Enzymol.
20   217:228-257 (1993); see, also, Scott and Smith, Science
249: 386-390 (1990), each of which is incorporated herein
by reference) describe methods of producing phage peptide
display libraries, including vectors and methods of
diversifying the population of peptides that are
25   expressed (see, also, Huse, WO 91/07141 and WO 91/07149,
each of which is incorporated herein by reference; see,
also, Example I). Phage display technology can be
particularly powerful when used, for example, with a
codon based mutagenesis method, which can be used to
30   produce random peptides or randomly or desirably biased
peptides (Huse, U.S. Patent No. 5,264,563, *supra*, 1993).
These or other well known methods can be used to produce
a phage display library, which can be subjected to the *in
vivo* panning method of the invention in order to identify

a peptide that homes to one or a few selected organs or
tissues.

In addition, to screening a phage display
library, *in vivo* panning can be used to screen various
5   other types of libraries, including, for example, an RNA
or cDNA library or a chemical library. If desired, the
organ homing molecule can be tagged, which can facilitate
recovery of the molecule from a selected organ or tissue
or identification of the molecule in the organ or tissue.
10 For example, where a library of organic molecules, each
containing a shared tag, is screened, the tag can be a
moiety such as biotin, which can be linked directly to
the molecule or can be linked to a support containing the
molecules. Biotin provides a shared tag useful for
15 recovering the molecule from a selected organ or tissue
using an avidin or streptavidin affinity matrix. In
addition, a molecule or a support containing a molecule
can be linked to a shared tag such as the hapten,
4-ethoxy-methylene-2-phenyl-2-oxazoline-5-one (phOx),
20 which can be bound by an anti-phOx antibody linked to a
magnetic bead as a means to recover the tagged molecule.
Methods for purifying biotin or phOx tagged molecules are
known in the art and the materials for performing these
procedures are commercially available (e.g., Invitrogen;
25 La Jolla CA; and Promega Corp.; Madison WI). In the case
where a phage library is screened, the phage can be
recovered using methods as disclosed in Example I.

In vivo panning provides a method for directly
identifying molecules that can home to one or a few
30 selected organs or tissues. As used herein, the term
"home" or "selectively home" means that a particular
molecule binds relatively specifically to a target
molecule present in one or few selected organs or tissues
following administration to a subject. In general,
35 selective homing is characterized, in part, by detecting

at least a two-fold (2x) greater specific binding of the
molecule to the selected organ or tissue as compared to a
control organ or tissue.

5   It should be recognized that, in some cases, a
molecule can localize nonspecifically to an organ or
tissue. For example, *in vivo* panning of a phage display
library resulted in a large number of phage localized in
organs such as liver, lung and spleen, which contain
components of the reticuloendothelial system (RES).
10   Selective homing in such tissues can be distinguished
from nonspecific binding by detecting differences, for
example, in the abilities of different individual phage
to home to a selected organ or tissue, including an organ
containing an RES component. For example, selective
15   homing can be identified by combining a putative organ
homing molecule such as a peptide expressed on a phage
with a large excess of non-infective phage or with about
a five-fold excess of phage expressing unselected
peptides, injecting the mixture into a subject and
20   collecting a sample of the selected organ or tissue. In
the latter case, for example, provided the number of
injected phage expressing organ homing peptides is
sufficiently low so as to be nonsaturating for the target
molecule, a determination that greater than 20% of the
25   phage in the selected organ or tissue express the
putative organ homing molecule is demonstrative evidence
that the peptide expressed by the phage is a specific
organ homing molecule. In addition, nonspecific
localization can be distinguished from selective homing
30   by performing competition experiments as described in
Examples II.D. and IV.

       Various methods can be useful for preventing
nonspecific binding of a molecule to an organ containing
a component of the RES. For example, a molecule that
35   homes selectively to such an organ can be obtained by

first blocking the RES using a material such as
polystyrene latex particles or dextran sulfate (see Kalin
et al., Nucl. Med. Biol. 20:171-174 (1993); Illum et al.,
J. Pharm. Sci. 75:16-22 (1986); Takeya et al., J. Gen.

5   Microbiol. 100:373-379 (1977); , each of which is
incorporated herein by reference), then administering the
library to the subject. Thus, Illum et al. administered
dextran sulfate 500 or polystyrene microspheres prior to
administration of a test substance to block nonspecific

10   uptake of the test substance by Kupffer cells, which are
the RES component of the liver (Illum et al., *supra*,
1986). In addition, Takeya et al. reported that the RES
in liver could be blocked using carbon particles, dextran
sulfate 500 (DS-500) or silica (Takeya et al., *supra*,

15   1977). Also, Kalin et al. reported that blockage of the
RES can be effected using a gelatine colloid (Kalin et
al., *supra*, 1993). These and various other agents useful
for blocking nonspecific uptake by the RES are known and
routinely used.

20   Undesired binding of phage to RES or to other
sites also can be prevented by coinjecting the mice with
a specific phage display library together with the same
phage made replication-deficient (see Smith et al.,
supra, 1990, 1993). In addition, a peptide that homes to

25   an organ containing an RES component can be identified by
preparing a phage display library using phage that
exhibit low background binding to such organs. For
example, Merrill et al. (Proc. Natl. Acad. Sci., *USA*
93:3188-3192 (1996), which is incorporated herein by

30   reference) selected lambda-type phage that are not taken
up by the RES and, as a result, remain in the circulation
for a prolonged period of time. A filamentous phage
variant can be selected using similar methods.

          As disclosed herein, uptake of peptide-

35   displaying phage by the RES in liver, for example, was

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blocked by coadministration of non-infective phage, which are not amplifiable. Phage expressing a CX_5 library were injected into mice, either alone or with an excess of non-infective phage (see Example II.E.).

5   Coadministration of non-infective phage with library phage substantially reduced the amount of phage recovered in liver and, to a lesser extent spleen, whereas little or no difference was observed in the number of phage recovered from brain, kidney or lung. These results

10 indicate that uptake of phage by organs such as liver and spleen, which, at least in part can be nonspecific due to the RES component of these organs, can be blocked by coadministration of non-infective phage with a peptide-displaying phage library.

15   Further experiments were performed to determine whether the phage that were present in liver upon coadministration with non-infective phage had selectively homed to the liver. A phage library was coadministered with non-infective phage, then phage recovered from the

20 liver after a first round of *in vivo* panning were amplified *in vitro* and used for a second round of *in vivo* panning (see Example II.E.). Phage were recovered from liver and from brain (control organ) and quantitated after each round of *in vivo* panning. More phage that

25 homed to liver as compared to brain were recovered following the second round of *in vivo* panning and the population of phage recovered from the liver following the first round of *in vivo* panning was enriched with phage that selectively home to liver. These results

30 demonstrate that the RES component of an organ such as liver can be blocked, thus allowing use of the *in vivo* panning method to identify a molecule that selectively homes to such an organ.

          As was observed for liver, a high amount of
35 phage localization also was observed in lung. However,

uptake of phage by lung is not nonspecific due, for
example, to uptake by alveolar phagocytes, which
constitute an RES component in lung. Specifically, phage
recovery essentially was the same regardless of whether a
5   phage library was injected alone or was coadministered
with non-infective phage (see Example II.E.). Thus, the
high uptake of phage observed in the lung (Pasqualini and
Ruoslahti, *supra*, 1996) likely is due to the large amount
of vasculature in the lungs.

10   Selective homing can be demonstrated by
determining the specificity of an organ homing molecule
for the selected organ or tissue as compared to a control
organ or tissue. For example, a ratio of brain:kidney
homing of up to 9:1 was observed for brain homing
15   peptides (i.e., 9x greater binding to the selected organ
as compared to the control organ; see Example II.A.).

             Selective homing also can be demonstrated by
showing that molecules that home to a selected organ, as
identified by one round of *in vivo* panning, are enriched
20   for organ homing molecules in a subsequent round of *in
vivo* panning. For example, phage expressing peptides
that selectively home to brain were isolated by *in vivo*
panning, then were subjected to additional rounds of *in
vivo* panning. As demonstrated in Example II.A., phage
25   recovered from brain after a first round of screening
showed an 8x enrichment in homing to brain as compared to
kidney following a second round of screening and a 13x
enrichment following a third round of *in vivo* panning.
When a peptide that selectively homes to brain was linked
30   to a moiety, i.e., a red blood cell (RBC), the
peptide/RBC complex selectively homed to brain (see
Example II.D.).

             Due to the conserved nature of cellular
receptors and of ligands that bind a particular receptor,

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the skilled artisan would recognize that an organ homing
molecule identified using, for example, *in vivo* panning
in a mouse also would bind to the corresponding target
molecule in the selected organ or tissue of a human or

5   other species. For example, an RGD-containing peptide
that can specifically bind to an integrin expressed by a
cell in a human subject also can bind integrins expressed
in a variety of species, including integrins expressed in
mammalian cells such as murine and bovine cells as well

10   as in cells of more evolutionarily distant species such
as *Drosophila*. The ability of an organ homing molecule
identified using *in vivo* panning in an experimental
animal such as a mouse readily can be examined for the
ability to bind to the corresponding organ or tissue in a

15   human subject by demonstrating, for example, that the
molecule also can bind specifically *in vitro* to a sample
of the selected organ or tissue obtained from a human
subject. Thus, routine methods can be used to confirm
that an organ homing molecule identified using *in vivo*

20   panning in an experimental animal also can bind an organ-
specific target molecule in a human subject.
Furthermore, such *in vitro* contacting of an organ homing
molecule with a sample suspected of containing a selected
organ or tissue can identify the presence of the selected

25   organ or tissue in the sample.

Furthermore, with regard to tumor homing
molecules, the skilled artisan would recognize that a
tumor homing molecule identified using, for example, *in
vivo* panning in a mouse having a murine tumor of a

30   defined histological type such as a melanoma also would
bind to the corresponding target molecule in a melanoma
in a human or other mammalian species. In addition, a
tumor homing molecule that binds a target molecule
present in the vasculature in a tumor grown in a mouse

35   likely also can bind to the corresponding target molecule
in the vasculature of a tumor in a human or other

mammalian subject. A tumor homing molecule identified
using *in vivo* panning in an experimental animal readily
can be examined for the ability to bind to a
corresponding tumor in a human patient by demonstrating,
5   for example, that the molecule also can bind specifically
in vitro to a sample of the tumor obtained from the
patient. Thus, routine methods can be used to confirm
that a tumor homing molecule identified using *in vivo*
panning in an experimental animal also can bind a target
10 molecule in a human tumor.

        The steps of administering the library to the
subject, collecting a selected organ or tissue and
identifying the molecules that home to the selected organ
or tissue, comprise a single round of *in vivo* panning.
15 Although not required, one or more additional rounds of
in vivo panning generally are performed. Where an
additional round of *in vivo* panning is performed, the
molecules recovered from the selected organ or tissue in
the previous round are administered to a subject, which
20 can be the same subject used in the previous round, where
only a part of the selected organ or tissue was
collected.

        By performing a second round of *in vivo*
panning, the relative binding selectivity of the
25 molecules recovered from the first round can be
determined by administering the identified molecules to a
subject, collecting the selected organ or tissue, and
determining whether more phage is recovered from the
organ or tissue as compared to the first round of
30 panning. In addition, if desired, a control organ or
tissue can be collected and used as a basis to compare
the molecules recovered from the selected organ with
those recovered from a control organ. An additional
round of *in vivo* panning also can indicate whether the
35 molecules identified from the initially selected organ or

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tissue also can home to additional organs or tissues, thus defining a family of selected organs or tissues. Additional rounds of panning can be performed as desired.

5   Ideally, no molecules are recovered from a control organ or tissue following a second or subsequent round of *in vivo* panning. Generally, however, a proportion of the molecules also will be present in a control organ or tissue. In this case, the ratio of molecules in the selected organ as compared to the

10   control organ (selected:control) can be determined. As described above, for example, phage that homed to brain following a first round of *in vivo* panning demonstrated a 13x enrichment in homing to the selected organ as compared to the control organ, kidney, following two

15   additional rounds of panning (Example II).

     Additional rounds of *in vivo* panning can be used to determine whether a particular molecule homes only to the selected organ or tissue or can recognize a target on the selected organ that also is expressed in

20   one or more other organs or tissues or is sufficiently similar to the target in the originally selected organ or tissue. Where a molecule is found to direct homing to organs or tissues in addition to the originally selected organ, the organs or tissues are considered to constitute

25   a family of selected organs or tissues. Using the method of *in vivo* panning, molecules that home to only a single selected organ or tissue and molecules that home to a family of selected organs or tissues can be defined. Such identification is expedited by collecting various

30   organs or tissues during subsequent rounds of *in vivo* panning.

     As used herein, the term "selected organ or tissue" is used in its broadest sense to mean an organ or a tissue to which a molecule selectively homes. In

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addition, the term "organ or tissue" is used broadly to
mean organ, tissue or cell type, including a cancer cell,
in which case the selected organ or tissue can be a tumor
such as a primary tumor or a metastatic lesion. In
5   general, a selected organ or tissue contains a cell that
expresses a particular target molecule such as a cell
surface receptor to which an organ homing molecule can
bind. By performing at least two rounds of *in vivo*
panning, the selectivity of homing of the molecule to the
10   selected organ or tissue can be determined (see
Example II). As discussed above, however, in some cases
an organ homing molecule can selectively home to more
than one selected organ or tissue, in which case the
molecule is considered to be able to selectively home to
15   a family of selected organs or tissues.

        The term "control organ or tissue" is used to
mean an organ or tissue other than a selected organ. A
control organ or tissue is characterized by the inability
of an organ homing molecule to home to the control organ
20   or tissue. A control organ or tissue is useful for
identifying nonspecific binding of a molecule. A control
organ or tissue can be collected, for example, to
identify nonspecific binding of the molecule or to
determine the selectivity of homing of the molecule (see
25   Examples I and II). In addition, nonspecific binding can
be identified by administering, for example, a control
molecule, which is known not to home to an organ or
tissue but is chemically similar to a potential organ
homing molecule. Alternatively, where the administered
30   molecules are linked to a support, administration of the
supports, alone, can be used to identify nonspecific
binding. For example, a phage that expresses the gene
III protein, alone, but that does not contain a peptide
fusion protein, can be screened by *in vivo* panning to
35   determine the level of nonspecific binding of the phage
support.

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In some cases it can take several rounds of *in
vivo* panning to identify a control organ or tissue, since
a molecule that selectively homes to an originally
selected organ also may have the ability to selectively
5 home to additional organs or tissues, thus defining a
family of selected organs or tissues. The ability of a
molecule to home to one or to a family of selected organs
or tissues allows for the preparation of panels of organ
homing molecules, wherein individual molecules variously
10 home to one selected organ or tissue or to any of a
family of selected organs or tissues with variable
selectivity.

In addition, it can be useful to determine
whether a molecule that selectively homes to a particular
15 type of tumor also selectively homes to the normal tissue
from which the tumor is derived. It can be desirable,
for example, to determine whether a molecule that homes
to a breast tumor also homes to normal breast tissue.
Thus, where *in vivo* panning is used to identify a tumor
20 homing molecule, a sample of the normal tissue
counterpart of the tumor can be examined to determine
whether homing of the molecule is selective for a target
molecule expressed only by the tumor cells or the
vascular tissue present in the tumor or is selective for
25 a target molecule normally expressed in the organ or
tissue from which the tumor is derived.

In general, a library of molecules, which
contains a diverse population of random or selectively
randomized molecules of interest, is prepared, then
30 administered to a subject. At a selected time after
administration, the subject is sacrificed and a selected
organ or tissue or part of the organ or tissue is
collected such that the molecules present in the selected
organ or tissue can be identified. For example, a mouse
35 was injected with a phage peptide display library, then,

after about 1 to 4 minutes, the mouse was anesthetized, frozen in liquid nitrogen to terminate circulation of the phage, a selected organ (brain or kidney; see Examples I and II) or tissue (breast tumor or melanoma; see

5   Example III) was collected, phage present in the selected organ were recovered and peptides that selectively homed to the selected organ or tissue were identified.

In the examples provided, the animals were sacrificed to collect the selected organ or tissue. It

10   should be recognized, however, that only a part of the selected organ need be collected to recover a molecule that homes to that organ. For example, a part of the selected organ or tissue can be collected by biopsy, such that a molecule such as a peptide expressed by a phage,

15   can be administered to the same subject a second time or more, as desired. Where the molecule that is to be administered a second time to the same subject is linked, for example, to a support or to a tag, the support or tag must be nontoxic and should be biodegradable, so as not

20   to interfere with subsequent rounds of screening.

In vitro screening of phage libraries previously has been used to identify peptides that bind to antibodies or to cell surface receptors (Smith and Scott, *supra*, 1993). For example, *in vitro* screening of

25   phage peptide display libraries has been used to identify novel peptides that specifically bound to integrin adhesion receptors (Koivunen et al., J. Cell Biol. 124:373-380 (1994a), which is incorporated herein by reference) and to the human urokinase receptor (Goodson

30   et al., Proc. Natl. Acad. Sci., USA 91:7129-7133 (1994)). However, such *in vitro* studies provide no insight as to whether a peptide that can specifically bind to a selected receptor *in vitro* also will bind the receptor *in vivo* or whether the binding peptide or the receptor are

unique to a specific organ in the body. Furthermore, the
in vitro methods are performed using defined,
well-characterized target molecules in an artificial
system. For example, Goodson et al. utilized cells

5   expressing a recombinant urokinase receptor. Thus, the
in vitro methods require prior knowledge of the target
molecule and yield little if any information regarding *in*
vivo utility. In contrast, the *in vivo* panning method
disclosed herein requires no prior knowledge or

10   availability of a target molecule and, therefore,
provides a significant advantage over previous methods by
identifying molecules that selectively home *in vivo* and
the target molecule present in an organ or tissue.

         Although a mechanism by which the disclosed

15   method of *in vivo* panning works has not been fully
defined, one possibility is that a molecule such as a
peptide expressed on a phage recognizes and binds to a
target molecule present on endothelial cells lining the
blood vessels in a selected organ. Evidence indicates,

20   for example, that the vascular tissues in various organs
differ from one another and that such differences can be
involved in regulating cellular trafficking in the body.
For example, lymphocytes home to lymph nodes or other
lymphoid tissues due, in part, to the expression of

25   specific "address" molecules by the endothelial cell in
those tissues (Salmi et al., *Proc. Natl. Acad. Sci., USA*
89:11436-11440 (1992)). Similarly, various leukocytes
can recognize sites of inflammation due, in part, to the
expression of endothelial cell markers induced by

30   inflammatory signals (see Butcher and Picker, *Science*
272:60-66 (1996); Springer, *Cell* 76:301-314 (1994)). In
addition, the endothelium present in kidney glomeruli
contains a binding protein for the atrial natriuretic
peptide (see Lewicki and Protter, in "Hypertension,

35   Pathophysiology, Diagnosis and Management" 2d ed (Raven
Press 1995), chapter 61). Thus, endothelial cell markers

provide a potential target for directing, for example, a
drug to a particular organ in a subject.

In some cases, the metastasis of cancer cells
to specific organs also can be due to recognition by the
5   tumor cell of an organ specific marker, including organ
specific endothelial cell markers (Fidler and Hart,
Science 217:998-1003 (1982)). The pattern of metastasis
of many cancers can be explained by assuming that
circulating tumor cells are preferentially trapped in the
10 first vascular bed encountered. Thus, the lungs and the
liver are the most frequent sites of cancer metastasis.
However, some cancers show patterns of metastasis that
are not explained by circulatory routing. Metastasis of
such cancers can be due, instead, to the presence of
15 selectively expressed address molecules such as
endothelial cell surface molecules expressed in the organ
to which the cancer metastasizes (see Goetz et al., Intl.
J. Canc. 65:192-199 (1996); Zhu et al., Proc. Natl. Acad.
Sci., USA 88:9568-9572 (1991); Pauli et al., Canc.
20 Metast. Rev. 9:175-189 (1990); Nicolson, Biochim.
Biophys. Acta 948:175-224 (1988)). The identification of
molecules that bind to such organ-specific endothelial
cell markers can provide a means to prevent tumor cell
metastasis to the particular organ.

25   Brain, kidney and two different experimental
tumors were selected as target organs or tissues to
identify phage expressing peptides that selectively home
to these selected organs or tissues because the blood
vessels in these organs have unique characteristics. For
30 example, the blood vessels in the brain form the
"blood-brain barrier" and express at least one specific
antigen (Schlosshauer and Herzog, J. Cell Biol. 110:1261-
1274 (1990)). In addition, the vasculature within a
tumor generally undergoes active angiogenesis, resulting
35 in the continual formation of new blood vessels to

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support the growing tumor. Such angiogenic blood vessels are distinguishable from mature vasculature in that angiogenic vasculature expresses unique endothelial cell surface markers, including the $\alpha_v\beta_3$ integrin (Brooks, Cell

5   79:1157-1164 (1994)) and receptors for angiogenic growth factors (Mustonen and Alitalo, J. Cell Biol. 129:895-898 (1995); Lappi, Semin. Canc. Biol. 6:279-288 (1995)). Moreover, tumor vasculature is histologically distinguishable from blood vessel in general in that

10   tumor vasculature is fenestrated (Folkman, Nat. Med. 1:27-31 (1995); Rak et al., Anticancer Drugs 6:3-18 (1995)). Thus, the unique characteristics of tumor vasculature make it a particularly attractive target for determining whether a molecule that homes specifically to

15   a tumor can be identified by *in vivo* panning. Such a tumor homing molecule can be useful for directing an agent such as a chemotherapeutic drug to a tumor, while reducing the likelihood the agent will have a toxic effect on normal, healthy organs or tissues. Moreover, a

20   molecule that homes selectively to tumor vasculature also may have use in targeting other types of neovasculature such as that present in inflammatory, regenerating or wounded tissues.

         Using *in vivo* panning, phage expressing various

25   peptides that selectively homed·to brain, to kidney, to a breast tumor or to a melanoma were identified (see Tables 1-4). Due to the large size of the phage (300 nm) and the short time the phage were allowed to circulate, it is unlikely that a substantial number of phage would

30   have exited the circulatory system, particularly in the brain and kidney. Thus, the organ homing molecules that were identified likely homed to and bound primarily endothelial cell surface markers that are expressed in an organ-specific manner (see, for example, Springer, Cell

35   76:301-314 (1994)). These results indicate that *in vivo*

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panning can be used to identify and analyze endothelial
cell specificities.

Immunohistochemical staining of brain following
administration of phage displaying the brain homing
5   peptide CLSSRLDAC (SEQ ID NO: 3) revealed staining within
the brain capillaries. No apparent preference for homing
of the phage to any particular region of the brain was
observed (see Pasqualini and Ruoslahti, *supra*, 1995).
Similarly, phage expressing the kidney homing peptide
10   CLPVASC (SEQ ID NO: 21) localized in kidney capillaries,
particularly in the glomeruli and between the tubules.
Such an analysis is not possible using endothelial cells
in culture because the cultured cells tend to lose their
tissue-specific differences (Pauli and Lee, Lab. Invest.
15   58:379-387 (1988)).

Although the conditions under which the *in vivo*
pannings were performed identified organ homing peptides
that generally bind to endothelial cell markers, specific
binding of phage expressing tumor homing peptides also
20   was observed in tumor parenchyma, indicating that target
molecules expressed on specific cells within an organ or
tissue also can be identified (see Example III). These
results indicate that phage expressing peptides can pass
through or leak from the blood vessels in the tumor,
25   possibly due to the fenestrated nature of the blood
vessels. Various normal organs such as liver also
contain vasculature that allows, for example, phage
expressing peptides to contact certain parenchymal cells.
Thus, *in vivo* panning can be useful for identifying
30   molecules that home to particular cells or tissue types
in an organ as well as to endothelial cells.

Phage peptide display libraries were
constructed essentially as described Smith and Scott
(*supra*, 1993; see, also, Koivunen et al., Biotechnology

13:265-270 (1995), which is incorporated herein by
reference). Oligonucleotides encoding peptides having
substantially random amino acid sequences were
synthesized based on an "NNK" codon, wherein "N" is A, T,
5   C or G and "K" is G or T. "NNK" encodes 32 triplets,
which encode the twenty amino acids and an amber STOP
codon (Scott and Smith, supra, 1990). At least one codon
encoding cysteine also was included in each
oligonucleotide so that cyclic peptides could be formed
10 through disulfide linkages (see Example I). The
oligonucleotides were inserted in frame with the sequence
encoding the gene III protein (gIII) in the vector fuse 5
such that a peptide-gIII fusion protein can be expressed.
Following expression, the fusion protein is expressed on
15 the surface of the phage containing the vector (Smith and
Scott, supra, 1993; Koivunen et al., Meth. Enzymol.
245:346-369 (1994b), which is incorporated herein by
reference).

        Remarkably, following in vivo panning, the
20 phage that selectively homed to brain, to kidney or to
tumors displayed only a few different peptide sequences
(see Tables 1-4 and Examples II and III). In some cases,
peptides having the same amino acid sequence were encoded
by phage having different oligonucleotide sequences
25 encoding the peptide. Furthermore, a family of brain
homing peptides was identified, wherein each peptide in
the family contained the common amino acid motif, SRL
(serine-arginine-leucine), but different flanking amino
acid sequences (see Table 1; SEQ ID NOS: 1, 3 and 5). In
30 addition, two different peptides displayed the motif VLR
(valine-leucine-arginine; see Table 1, SEQ ID NOS: 4 and
16). These results demonstrate that it is the peptide
displayed by the phage, rather than some incidental
mutant property of the phage, that directs homing to the
35 selected organ or tissue.

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The sequences of the brain, kidney or tumor
homing motifs do not reveal any significant similarities
with known ligands for endothelial cell receptors nor do
they resemble any sequences listed in various data banks.
5   However, some of the brain-homing motifs share a
similarity with integrin-binding sequences. For example,
one brain homing peptide contained an RLD sequence, which
is recognized by certain integrins (Altieri et al.,
J. Biol. Chem. 265:12119-12122 (1990); Koivunen et al.,
10   _supra_, 1994a), and the DXXR (SEQ ID NO: 44) motif in
another peptide resembles the RGD, DGR, and NGR motifs
that bind to certain integrins (Ruoslahti, _J. Clin._
Invest. 87:1-5 (1991); Koivunen et al., _supra_, 1994a).

In vivo panning also was used to identify
15   molecules that selectively home to a tumor. Mice bearing
a breast tumor were injected with a phage library and
phage that homed to the breast tumor were recovered and
the peptides for 14 phage were determined (see Example
III.A.). The 14 phage expressed four different peptides,
20   including the peptide CGRECPRLCQSSC (SEQ ID NO: 48),
which was expressed by six of the 14 phage.

The general applicability of the _in vivo_
panning method for identifying molecules that home to a
tumor was examined by injecting mice bearing a syngeneic
25   melanoma with phage expressing a diverse population of
peptides (see Example III.B.). The B16 mouse melanoma
model was selected for these studies because the tumors
that form are highly vascularized and because the biology
of this tumor line has been thoroughly characterized (see
30   Miner et al., _Cancer Res._ 42:4631-4638 (1982)).
Furthermore, because the B16 melanoma cells are of mouse
origin, species differences between the host and the
tumor cell donor will not affect, for example, the
distribution of phage into the tumor as compared to into
35   normal organs.

About three-fold enrichment of phage homing
into the tumor relative to brain (control organ) was
obtained. In particular, two peptides, WGTGLC (SEQ ID
NO: 52) and CLSGSLSC (SEQ ID NO: 55), represented greater

5   than 75% of the peptides expressed on phage that were
recovered from the tumor and sequenced (see
Example III.B.). Immunohistochemical staining of tumor
and other organs using an anti-phage antibody
demonstrated that the CLSGSLSC (SEQ ID NO: 50) expressing

10   phage produced strong staining in the melanoma, but
essentially no staining in skin or in kidney (control
organs). The staining pattern generally followed the
blood vessels within the melanoma, but was not strictly
confined to the blood vessels. These results indicate

15   that tumor homing molecules can be identified by *in vivo*
panning and that such molecules can home to vascular
tissue in the tumor as well as to tumor parenchyma,
probably due to the fenestrated nature of the blood
vessels permitting ready exit of the phage from the

20   circulatory system.

The identification of brain homing peptides and
breast tumor homing peptides that contain potential
integrin binding motifs such as "RE" and "NGR" indicates
that *in vivo* panning can be useful to identify integrin

25   binding peptides (see Koivunen et al., *supra*, 1995). The
$\alpha_v\beta_3$ integrin, for example, is expressed in blood vessels
undergoing angiogenesis but not in quiescent endothelial
cells. Binding of a peptide or antibody antagonist to
the $\alpha_v\beta_3$ integrin induces apoptosis of the angiogenic

30   vessels, possibly by interrupting a signal transduced
into the proliferating endothelial cells as a result of
$\alpha_v\beta_3$ integrin binding to the extracellular matrix (Brooks
et al., *supra*, 1994).

Since the $\alpha_v\beta_3$ integrin is expressed by

35   endothelial cells in angiogenic vasculature, experiments

were performed to determine whether tumor vasculature
that is undergoing angiogenesis can be targeted *in vivo*
using methods as disclosed herein. Phage expressing the
peptide, CDCRGDCFC (SEQ ID NO: 57; "RGD phage;" see,

5   Koivunen et al., *supra*, 1995), which is known to bind to
the $\alpha_v\beta_3$ integrin, were injected into mice bearing tumors
formed from human breast carcinoma cells, human melanoma
cells or mouse melanoma cells (see Example IV). The RGD
phage selectively homed to each of the tumors, whereas

10   such homing did not occur with control phage. For
example, in mice bearing tumors formed by implantation of
human breast carcinoma cells, a twenty- to eighty-fold
greater number of the RGD phage, as compared to
unselected control phage, accumulated in the tumor.

15   Tissue staining for the phage showed
accumulation of the RGD phage in the blood vessels within
the tumor, whereas no staining was observed in brain or
kidney (control organs). Specificity of tumor homing of
the RGD phage was demonstrated by competition

20   experiments, in which coinjection of the RGD-containing
peptide greatly reduced tumor homing of the RGD phage,
whereas coinjection of a non-RGD-containing control
peptide had no effect on homing of the RGD phage (see
Example IV). These results demonstrate that the $\alpha_v\beta_3$

25   target molecule is expressed on the luminal surface of
endothelial cells in a tumor and that a peptide that
binds to an α_v-containing integrin can bind selectively to
this integrin and, therefore, to vasculature undergoing
angiogenesis. In view of these results, the skilled

30   artisan would recognize that an RGD peptide or an
antibody that binds an α_v integrin, such as the monoclonal
antibody LM609, which binds an $\alpha_v\beta_3$ integrin (see Brooks
et al., *supra*, 1994), can be used to target an organ or
tissue containing angiogenic vasculature, including a

35   tumor containing angiogenic vasculature.

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The ability of a molecule that homes to a
particular tumor to selectively home to another tumor of
the same or a similar histologic type can be determined
using, for example, human tumors grown in nude mice or
5   mouse tumors grown in syngeneic mice for these
experiments. For example, various human breast cancer
cell lines, including MDA-MB-435 breast carcinoma (Price
et al., Cancer Res. 50:717-721 (1990)), SKBR-1-II and SK-
BR-3 (Fogh et al., J. Natl. Cancer Inst. 59:221-226
10 (1975)), and mouse mammary tumor lines, including EMT6
(Rosen et al., Intl. J. Cancer 57:706-714 (1994)) and
C3-L5 (Lala and Parhar, Intl. J. Cancer 54:677-684
(1993)), are readily available and commonly used as
models for human breast cancer. Using such breast tumor
15 models, for example, information relating to the
specificity of an identified breast tumor homing molecule
for diverse breast tumors can be obtained and molecules
that home to a broad range of different breast tumors or
provide the most favorable specificity profiles can be
20 identified. In addition, such analyses can yield new
information, for example, about tumor stroma, since
stromal cell gene expression, like that of endothelial
cells, can be modified by the tumor in ways that cannot
be reproduced *in vitro*.

25   Selective homing of a molecule such as a
peptide or protein to a selected organ or tissue can be
due to specific recognition by the peptide of a
particular cell target molecule such as a cell surface
receptor present on a cell in the organ or tissue.
30 Selectivity of homing is dependent on the particular
target molecule being expressed on only one or a few
different cell types, such that the molecule homes to
only one or a few organs or tissues. As discussed above,
the identified brain, kidney and, at least in part, tumor
35 homing peptides likely are recognizing endothelial cell
surface markers in the blood vessels present in these

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organs or tissues. However, most different cell types,
particularly cell types that are unique to an organ or
tissue, can express unique target molecules. Thus, in
organs such as liver, spleen or lymph node, where blood
5 circulates through sinusoids formed by the cells specific
for the organ, *in vivo* panning can be useful for
identifying molecules that home to the particular organ.

An organ homing molecule such as the peptides
exemplified in Tables 1-4 can be used to direct a moiety
10 to a selected organ or tissue by linking the moiety to
the molecule. As used herein, the term "moiety" is used
broadly to mean a physical, chemical, or biological
material that is linked to an organ homing molecule. For
example, a moiety can be an agent such as a drug or can
15 be a chambered microdevice, which can contain an agent.
In addition, a moiety can be a molecule such as a protein
or nucleic acid, to which an organ homing molecule is
grafted for the purpose of directing the protein or
nucleic acid to a selected organ or tissue. For example,
20 a peptide organ homing molecule can be expressed as a
fusion protein with a desired protein such that the
peptide directs the protein to a selected organ.

A moiety can be a detectable label such a
radiolabel or can be a cytotoxic agent, including a toxin
25 such as ricin or a drug such as a chemotherapeutic agent
or can be a physical, chemical or biological material
such as a liposome, microcapsule, micropump or other
chambered microdevice, which can be used, for example, as
a drug delivery system. Generally, such microdevices,
30 should be nontoxic and, if desired, biodegradable.
Various moieties, including microcapsules, which can
contain an agent, and methods for linking a moiety or
chambered microdevice to a molecule of the invention are
well known in the art and commercially available (see,
35 for example, "Remington's Pharmaceutical Sciences" 18th

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ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow
and Lane, <u>Antibodies: A laboratory manual</u> (Cold Spring
Harbor Laboratory Press 1988), each of which is
incorporated herein by reference; see, also, Hermanson,
5   *supra*, 1996).

        Linking of a moiety to an organ homing molecule
for the purpose of directing homing of the moiety to the
selected organ or tissue is exemplified by the linking of
a brain homing peptide to a RBC, wherein the peptide
10 directed homing of the RBC to brain (see Example II.D.).
These results indicate that an organ homing molecule of
the invention can be linked to other cell types or to a
physical, chemical or biological delivery system such as
a liposome or other encapsulating device, which can
15 contain an agent such as drug, in order to direct the
cell type or the delivery system to a selected organ or
tissue. For example, a tumor homing molecule identified
by *in vivo* panning can be linked to a white blood cell
(WBC) such as a cytotoxic T cell or a killer cell,
20 wherein upon administration of the tumor homing
molecule/WBC complex, the molecule directs homing of the
WBC to the tumor, where the WBC can exert its effector
function. Similarly, an organ homing molecule can be
linked to a liposome or to a microcapsule comprising, for
25 example, a permeable or semipermeable membrane, wherein
an agent such as a drug to be delivered to a selected
organ or tissue is contained within the liposome or
microcapsule.

        In one embodiment, an organ homing molecule is
30 linked to a moiety that is detectable external to the
subject in order to perform an *in vivo* diagnostic imaging
study. For example, *in vivo* imaging using a detectably
labeled brain homing peptide can identify a region in the
brain where circulation is occluded. For such studies, a
35 gamma ray emitting radionuclide such as indium-111 or

technitium-99 can be linked to a brain homing molecule
and, following administration to a subject, can be
detected using a solid scintillation detector.
Alternatively, a positron emitting radionuclide such as
5   carbon-11 or a paramagnetic spin label such as carbon-13
can be linked to the molecule and, following
administration to a subject, the localization of the
moiety/molecule can be detected using positron emission
transaxial tomography or magnetic resonance imaging,
10 respectively.

        Such *in vivo* imaging methods also can be used
to identify the presence of cancer in a subject by
linking an appropriate moiety to a tumor homing molecule,
which can recognize a unique target expressed by the
15 tumor cells or by the blood vessels formed by
angiogenesis in the tumor. Such methods can identify a
primary tumor as well as a metastatic lesion, which may
not be detectable using other methods. Having identified
the presence of such cancer, in another embodiment of the
20 invention, the tumor homing molecule can be linked to a
cytotoxic agent such as ricin or a cancer
chemotherapeutic agent in order to direct the moiety to
the tumor or can be linked to a chambered microdevice,
which can contain, for example, a chemotherapeutic drug
25 or other cytotoxic agent. Such a method can allow
selective killing of the tumor, while substantially
sparing normal tissues.

        When administered to a subject, the
molecule/moiety complex is administered as a
30 pharmaceutical composition containing, for example, the
complex and a pharmaceutically acceptable carrier.
Pharmaceutically acceptable carriers are well known in
the art and include, for example, aqueous solutions such
as water or physiologically buffered saline or other

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solvents or vehicles such as glycols, glycerol, oils such
as olive oil or injectable organic esters.

A pharmaceutically acceptable carrier can
contain physiologically acceptable compounds that act,
5   for example, to stabilize or to increase the absorption
of the complex. Such physiologically acceptable
compounds include, for example, carbohydrates, such as
glucose, sucrose or dextrans, antioxidants, such as
ascorbic acid or glutathione, chelating agents, low
10 molecular weight proteins or other stabilizers or
excipients. One skilled in the art would know that the
choice of a pharmaceutically acceptable carrier,
including a physiologically acceptable compound, depends,
for example, on the route of administration of the
15 composition. The pharmaceutical composition also can
contain an agent such as a cancer therapeutic agent.

One skilled in the art would know that a
pharmaceutical composition containing an organ homing
molecule can be administered to a subject by various
20 routes including, for example, orally or parenterally,
such as intravenously. The composition can be
administered by injection or by intubation. The
pharmaceutical composition also can be an organ homing
molecule linked to liposomes or other polymer matrices,
25 which can have incorporated therein, for example, a drug
such as a chemotherapeutic agent (Gregoriadis, Liposome
Technology, Vol. 1 (CRC Press, Boca Raton, FL 1984),
which is incorporated herein by reference). Liposomes,
for example, which consist of phospholipids or other
30 lipids, are nontoxic, physiologically acceptable and
metabolizable carriers that are relatively simple to make
and administer.

For the diagnostic or therapeutic methods
disclosed herein, an effective amount of the

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molecule/moiety complex must be administered to the
subject. As used herein, the term "effective amount"
means the amount of the complex that produces the desired
effect. An effective amount often will depend on the
5   moiety linked to the organ homing molecule. Thus, a
lesser amount of a radiolabeled molecule can be required
for imaging as compared to the amount of a drug/molecule
complex administered for therapeutic purposes. An
effective amount of a particular molecule/moiety for a
10 specific purpose can be determined using methods well
known to those in the art.

The route of administration of an organ homing
molecule will depend, in part, on the chemical structure
of the molecule. Peptides, for example, are not
15 particularly useful when administered orally because they
can be degraded in the digestive tract. However, methods
for chemically modifying peptides to render them less
susceptible to degradation by endogenous proteases or
more absorbable through the alimentary tract are well
20 known (see, for example, Blondelle et al., *supra*, 1995;
Ecker and Crooke, *supra*, 1995; Goodman and Ro, *supra*,
1995). Such methods can be performed on peptides
identified by *in vivo* panning. In addition, methods for
preparing libraries of peptide analogs such as peptides
25 containing D-amino acids; peptidomimetics consisting of
organic molecules that mimic the structure of peptide; or
peptoids such as vinylogous peptoids, are known in the
art and can be used to identify molecules that home to a
selected organ or tissue and are stable for oral
30 administration.

Organ homing molecules obtained using the
methods disclosed herein also can be useful for
identifying the presence of a target molecule such as a
cell surface receptor or a ligand for a receptor, which
35 is recognized by the organ homing peptide, or for

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substantially isolating the target molecule. For
example, an organ homing peptide can be linked to a solid
support such as a chromatography matrix. The linked
peptide then can be used for affinity chromatography by
5   passing an appropriately processed sample of a selected
organ or tissue over the column in order to bind a
particular target molecule. The target molecule, which
forms a complex with the organ homing molecule, then can
be eluted from the column and collected in a
10   substantially isolated form. The substantially isolated
target molecule can be characterized using well known
methods. An organ homing molecule also can be linked to
a detectable moiety such as a radionuclide, a fluorescent
molecule, an enzyme or biotin and can be used, for
15   example, to screen a sample in order to detect the
presence of the target molecule or to follow the target
molecule during various isolation steps.

The methods of the present invention were used
to identify peptides that selectively home to a selected
20   organ or tissue. Thus, the present invention also
provides organ homing peptides, including brain homing
peptides such as CNSRLHLRC (SEQ ID NO: 1), CENWWGDVC (SEQ
ID NO: 2), WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) and
others as shown in Table 1; kidney homing peptides such
25   as CLPVASC (SEQ ID NO: 21), CGAREMC (SEQ ID NO: 22) and
others as shown in Table 2; and tumor homing peptides
such as the breast tumor homing peptides CGRECPRLCQSSC
(SEQ ID NO: 48) and others as shown in Table 3; and the
melanoma homing peptides WGTGLC (SEQ ID NO: 52), CLSGSLSC
30   (SEQ ID NO: 55) and others as shown in Table 4.

It should be recognized that cysteine residues
were included in the peptides such that cyclization of
the peptides could be effected. In fact, the peptides
containing at least two cysteine residues cyclize
35   spontaneously. However, such cyclic peptides also can be

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active when present in a linear form (see, for example, Koivunen et al., _J. Biol. Chem._ 268:20205-20210 (1993)). Thus, in some cases one or both of the cysteine residues in a peptide can be deleted without significantly

5 affecting the organ homing activity of a peptide of the invention. For example, a peptide having the sequence LSSRLDA (SEQ ID NO: 19; compare SEQ ID NO: 3) also can be a brain homing peptide. Similarly, the amino acid residues N-terminal and C-terminal to the first and last

10 cysteine residues, respectively, in a peptide such as WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) can be dispensable without substantially altering brain homing activity of the peptide. Thus, a peptide having the sequence VLREGPAGG (SEQ ID NO: 20) also can be useful as a brain

15 homing peptide. Methods for determining the necessity of a cysteine residue or of amino acid residues N-terminal or C-terminal to a cysteine residue for organ homing activity of a peptide of the invention are routine and well known in the art.

20 An organ homing peptide is useful, for example, for targeting a desired moiety to the selected organ or tissue as discussed above. In addition, an organ homing peptide can be used to identify the presence of a target molecule in a sample. As used herein, the term "sample"

25 is used in its broadest sense to mean a cell, tissue, organ or portion thereof that is isolated from the body. A sample can be, for example, a histologic section or a specimen obtained by biopsy or cells that are placed in or adapted to tissue culture. If desired, a sample can

30 be processed, for example, by homogenization, which can be an initial step for isolating the target molecule to which an organ homing molecule binds.

An organ homing peptide such as a brain homing peptide can be used to identify the target molecule

35 expressed in brain. For example, a brain homing peptide

can be attached to a matrix such as a chromatography
matrix to produce a peptide affinity matrix. A
homogenized sample of brain can be applied to the
peptide-affinity matrix under conditions that allow
5   specific binding of the brain homing peptide to the
target molecule (see, for example, Deutshcer, Meth.
Enzymol., Guide to Protein Purification (Academic Press,
Inc., ed. M.P. Deutscher, 1990), Vol. 182, which is
incorporated herein by reference; see, for example, pages
10   357-379). Unbound and nonspecifically bound material can
be removed and the specifically bound brain-derived
target molecule can be isolated in substantially purified
form.

The disclosed *in vivo* panning method can be
15   used to detect four different kinds of target molecules
in tumors. First, because tumor vasculature undergoes
active angiogenesis, target molecules that are
characteristic of angiogenic vasculature, in general, or
angiogenic tumor vasculature, in particular, can be
20   identified. Second, vascular target molecules that are
characteristic of the tissue of origin of the tumor can
be identified. Third, target molecules that are
expressed in the vasculature of a particular type of
tumor can be identified. Fourth, tumor stroma or tumor
25   cell target molecules can be identified due to the
fenestrated nature of tumor vasculature, which allows the
potential organ homing molecules to leave the circulation
and contact the tumor parenchyma.

As an alternative to using an organ or tissue
30   sample, extracts of cultured endothelial cells can be
used as the starting material in order to enhance the
concentration of the receptor in the sample. For
example, brain-derived endothelial cell lines such as
BEND cells (Montesano et al., Cell 62:435-445 (1990),
35   which is incorporated herein by reference) can be

examined for the expression of a particular receptor for
a brain homing peptide (see, also, PCT/FR95/01313, filed
October 9, 1995, which is incorporated herein by
reference). The presence of the receptor can be
5 established by using phage binding and cell attachment
assays (see, for example, Barry et al., Nat. Med.
2:299-305 (1996), which is incorporated herein by
reference).

A cell line expressing a particular receptor
10 can be identified and surface iodination of the cells can
be used to label the receptor. The cells then can be
extracted with octylglucoside and the extract can be
fractionated by affinity chromatography using a brain
homing peptide (see Table 1) coupled to a matrix such as
15 Sepharose™. Extracts prepared from HUVEC cells (human
umbilical vein endothelial cells) can be used as a
control for the brain-derived endothelial cells. The
purified receptor can be microsequenced and antibodies
can be prepared. If desired, oligonucleotide probes can
20 be prepared and used to isolate cDNA clones encoding the
receptor. Alternatively, an anti-receptor antibody can
be used to isolate a cDNA clone from an expression
library (see Argraves et al., J. Cell Biol. 105:1183-1190
(1987), which is incorporated herein by reference).

25   As an alternative to isolating the receptor, a
nucleic acid encoding the receptor can be isolated using
a mammalian cell expression cloning system such as the
COS cell system. An appropriate library can be prepared,
for example, using mRNA from primary brain endothelial
30 cells (Lathey et al., Virology 176:266-273 (1990), which
is incorporated herein by reference). The nucleic acids
can be cloned into the pcDNAI vector (Invitrogen), for
example. Cells expressing a cDNA for the receptor can be
selected by binding to the brain homing peptide.
35 Purified phage can be used as the carrier of the peptide

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and can be attached to magnetic beads coated, for
example, with anti-M13 antibodies (Pharmacia). Cells
that bind to the peptide coating can be recovered using a
magnet and the plasmids can be isolated. The recovered
5 plasmid preparations then can be divided into pools and
examined in COS cell transfections. The procedure can be
repeated until single plasmids are obtained that can
confer upon the COS cells the ability to bind the brain
homing peptide.

10   The following examples are intended to
illustrate but not limit the present invention.

EXAMPLE I
IN VIVO PANNING

          This example demonstrates methods for preparing
15 a phage library and screening the library using *in vivo*
panning to identify phage expressing peptides that home
to a selected organ or tissue.

A. Preparation of phage libraries:

          Phage display libraries were constructed using
20 the fuse 5 vector as described by Koivunen et al. (*supra*,
1995; see, also, Koivunen et al., *supra*, 1994b)). Six
libraries encoding peptides designated CX_5C (SEQ ID NO:
36), CX_6C (SEQ ID NO: 37), CX_7C (SEQ ID NO: 38), CX, (SEQ
ID NO: 39), $X_2CX_{14}CX_2$ (SEQ ID NO: 40) and X_2CX_{18} (SEQ ID NO:
25 41) were prepared, where "C" indicates cysteine and "X_N"
indicates the given number of individually selected amino
acids. These libraries can display cyclic peptides when
at least two cysteine residues are present in the
peptide.

30   Oligonucleotides were constructed such that "C"
was encoded by the codon TGT and "X_N" was encoded by NNK,

where "N" is equal molar mixtures of A, C, G and T, and
where "K" is equal molar mixtures of G and T. Thus, the
peptide represented by CX$_5$C (SEQ ID NO: 36) can be
represented by an oligonucleotide having the sequence

5   TGT(NNK)$_5$TGT (SEQ ID NO: 42). Oligonucleotides were made
double stranded by 3 cycles of PCR amplification,
purified and ligated to the nucleic acid encoding the
gene III protein in the fuse 5 vector such that, upon
expression, the peptide is present as a fusion protein at

10 the N-terminus of the gene III protein.

The vectors were transfected by electroporation
into MC1061 cells. Bacteria were cultured for 24 hr in
the presence of 20 μg/ml tetracycline, then phage were
collected from the supernatant by precipitation twice

15 using polyethylene glycol. Each library contained about
5 x 10^9 to 5 x 10^{14} transducing units (TU; individual
recombinant phage).

B.   In vivo panning of phage:

A mixture of phage libraries containing 1 x 10^{14}

20 TU was diluted in 200 μl DMEM and injected into the tail
vein of anesthetized BALB\c mice (2 month old females;
Jackson Laboratories; Bar Harbor ME); Avertin^{TM}
(0.015 ml/g) was used as anesthetic (see Pasqualini and
Ruoslahti, *supra*, 1996). After 1-4 minutes, mice were

25 snap frozen in liquid nitrogen. To recover the phage,
carcasses were partially thawed at room temperature for 1
hr, organs were collected and weighed, then were ground
in 1 ml DMEM-PI (DMEM containing protease inhibitors
(PI); phenylmethylsulfonyl fluoride (PMSF; 1 mM),

30 aprotinin (20 μg/ml), leupeptin (1 μg/ml)).

Organ samples were washed 3 times with ice cold
DMEM-PI containing 1% bovine serum albumin (BSA), then
directly incubated with 1 ml K91-kan bacteria for 1 hr.

50

Ten ml NZY medium containing 0.2 µg/ml tetracycline
(NZY/tet) was added to the bacterial culture, the mixture
was incubated in a 37°C shaker for 1 hr, then 200 µl
aliquots were plated in agar plates containing 40 µg/ml
5   tetracycline (tet/agar).

    Individual colonies containing phage recovered
from brain or kidney were grown for 16 hr in 5 ml
NZY/tet. The bacterial cultures obtained from the
individual colonies were pooled and the phage were
10   purified and re-injected into mice as described above for
a second round of *in vivo* panning. A third round of
panning also was performed. Phage DNA was purified from
individual bacterial colonies obtained from the second
and the third round of *in vivo* panning and the DNA
15   sequences encoding the peptides expressed by selected
phage were determined (see Koivunen et al., *supra*,
1994b).

EXAMPLE II
CHARACTERIZATION OF PEPTIDES THAT HOME
20   TO A SELECTED ORGAN

    This example demonstrates that an organ homing
peptide of the invention selectively homes to a selected
organ and that an organ homing .peptide identified by *in
vivo* panning can be used to direct a moiety to a selected
25   organ.

A. Brain is the selected organ

    Three rounds of *in vivo* panning in mice were
performed. Kidney was used as a control organ. Mice
were injected with two different mixtures of phage
30   libraries. The first mixture contained libraries
encoding CX_7 (SEQ ID NO: 39), CX_5C (SEQ ID NO: 36), CX_6C
(SEQ ID NO: 37) and CX_7C (SEQ ID NO: 38) peptides
(CX_{5-7}/CX_7 mixture; SEQ ID NOS: 36-39). The second mixture

contained libraries encoding $X_2CX_{14}CX_2$ (SEQ ID NO: 40) and
X_2CX_{18} (SEQ ID NO: 41) peptides ($X_2CX_{18}/X_2CX_{14}CX_2$ mixture;
SEQ ID NOS: 40 and 41).

5   The phage library mixtures were administered to
mice via tail vein injection. Phage input was 1×10^{16} TU
of the CX_{5-7}/CX_9 (SEQ ID NOS: 36-39) mixture or 1×10^{14} TU
of the $X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) mixture.
Phage were recovered from the brains of the injected
mice, the recovered phage were amplified in vitro, then a
10 second and a third round of in vivo panning was
performed. During the second and third rounds of
panning, phage were recovered from brain and from kidney
and the number of TU from each organ was compared. This
comparison revealed that 6x more phage from the CX_{5-7}/CX_9
15 (SEQ ID NOS: 36-39) mixture bound to brain than to kidney
in the second round and 13x more of the CX_{5-7}/CX_9 (SEQ ID
NOS: 36-39) phage bound to brain than to kidney in the
third round of panning. Following administration of the
$X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) mixture, an 11x and
20 8x enrichment of phage homing to the brain as compared to
kidney occurred during the second and third rounds of
panning, respectively. Thus, substantial enrichment of
phage binding to the brain was observed following the
second and third rounds of in vivo panning.

25 The amino acid sequences were determined for
the inserts present in 73 cloned phage that were
recovered from brain during the second and third rounds
of in vivo panning. Peptides containing an SRL motif
predominated (36% of the clones sequenced; see SEQ ID
30 NOS: 1, 3 and 5), followed by peptides containing the VLR
motif (20.5% of the clones; see SEQ ID NOS: 4 and 16) and
the peptide CENWWGDVC (SEQ ID NO: 2; 19% of the clones;
see Table 1). Other peptides that occurred much less
frequently, but were present more than once, included
35 CGVRLGC (SEQ ID NO: 6), CKDWGRIC (SEQ ID NO: 7), CLDWGRIC

(SEQ ID NO: 8) and CTRITESC (SEQ ID NO: 9). Eight other
sequences appeared only one time each and were not
characterized further. The SRL tripeptide motif was
present in several different sequence contexts,

5   indicating that the nucleic acids encoding the peptides
were derived from a number of independent phage. These
results indicate that the selection of the peptides
containing the SRL motif represents the specific binding
of several independent phage displaying peptides with the

10   SRL sequence and is not an artifact due, for example, to
phage amplification. In addition, in some cases,
different phage expressed peptides that had the same
amino acid sequence, but were encoded by oligonucleotides
having different sequences, thus confirming that homing

15   of a particular phage to an organ is due to the specific
peptide expressed on the phage.

        To determine the specificity of brain homing of
the individual motifs identified, phage displaying the
predominant motifs were amplified individually, diluted

20   to the same input titer and administered to mice.
Following administration, brain and kidney were removed
and the number of TU of phage in each organ was
determined. The enrichment ratio of phage recovered from
the selected organ, brain, as compared to the control

25   organ, kidney, revealed that phage displaying one of the
four most recovered peptides, CNSRLHLRC (SEQ ID NO: 1),
CENWWGDVC (SEQ ID NO: 2), WRCVLREGPAGGCAWFNRHRL (SEQ ID
NO: 16), CLSSRLDAC (SEQ ID NO: 3), each selectively
targeted the brain as compared to the kidney.

30   Specifically, the ratio of selective homing
(brain:kidney) was about 8 for CNSRLHLRC (SEQ ID NO: 1)
and for CLSSRLDAC (SEQ ID NO: 3), 4 for CENWWGDVC (SEQ ID
NO: 2) and 9 for WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16).

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TABLE 1

PEPTIDES FROM PHAGE RECOVERED FROM BRAIN

A.A. SEQUENCE	# PHAGE DISPLAYING SAME A.A. SEQUENCE (% PHAGE)
$CX_{5-7}C/CX_9$ (36-39)* library:	
CNSRLHLRC (1)*	16 (21.9%)
CENWWGDVC (2)	14 (19.2%)
CLSSRLDAC (3)	6 (8.2%)
CVLRGGRC (4)	5 (6.8%)
CNSRLQLRC (5)	4 (5.5%)
CGVRLGC (6)	3 (4.1%)
CKDWGRIC (7)	2 (2.8%)
CLDWGRIC (8)	2 (2.8%)
CTRITESC (9)	2 (2.8%)
CETLPAC (10)	1 (1.4%)
CRTGTLFC (11)	1 (1.4%)
CGRSLDAC (12)	1 (1.4%)
CRHWFDVVC (13)	1 (1.4%)
CANAQSHC (14)	1 (1.4%)
CGNPSYRC (15)	1 (1.4%)
$X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) library:	
WRCVLREGPAGGCAWFNRHRL (16)	10 (13.7%)
YPCGGEAVAGVSSVRTMCSE (17)	1 (1.4%)
LNCDYQGTNPATSVSVPCTV (18)	1 (1.4%)

* - numbers in parentheses indicate SEQ ID NO:.

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Two control phage showed low binding to both brain and
kidney. These results demonstrate that *in vivo* panning
can be used to screen phage display libraries in order to
identify phage expressing peptides that home to a
5 selected organ.

B. Kidney as the selected organ

The same methodology used to isolate phage
expressing brain homing peptides was used to isolate
phage expressing peptides that home to kidney. In these
10 experiments, brain was used as the control organ. A
mixture of the CX_5C (SEQ ID NO: 36) and the CX_6C (SEQ ID
NO: 37) libraries was administered as described above.
Homing of phage to the kidney was obtained and an
approximately 3x to 7x enrichment of phage homing to
15 kidney was observed following a second round of *in vivo*
panning.

The amino acid sequences were determined for
the inserts in 48 cloned phage that homed to kidney. The
peptides expressed by these phage were represented by two
20 predominant sequences, CLPVASC (SEQ ID NO: 21; 46% of the
clones sequenced) and CGAREMC (SEQ ID NO: 22; 17% of the
clones; see Table 2). In addition, the peptide CKGRSSAC
(SEQ ID NO: 23) appeared three times and three other
peptides were present twice each. Phage expressing a
25 CLPVASC (SEQ ID NO: 21), CGAREMC (SEQ ID NO: 22) or
CKGRSSAC (SEQ ID NO: 23) peptide each exhibited selective
homing to kidney; the ratio of selective kidney:brain
homing was 7 for CLPVASC (SEQ ID NO: 21), 3 for CGAREMC
(SEQ ID NO: 22) and 2 for CKGRSSAC (SEQ ID NO: 23).

30 These results demonstrate that the *in vivo*
panning method is a generally applicable method for
screening a phage library to identify phage expressing
peptides that home to a selected organ. Database

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TABLE 2
PEPTIDES FROM PHAGE RECOVERED FROM KIDNEY

A.A. SEQUENCE		# PHAGE DISPLAYING SAME A.A. SEQUENCE (% PHAGE)
CLPVASC	(21)*	22 (45.8%)
CGAREMC	(22)	8 (16.7%)
CKGRSSAC	(23)	3 (6.2%)
CWARAQGC	(24)	2 (4.2%)
CLGRSSVC	(25)	2 (4.2%)
CTSPGGSC	(26)	2 (4.2%)
CMGRWRLC	(27)	1 (2.1%)
CVGECGGC	(28)	1 (2.1%)
CVAWLNC	(29)	1 (2.1%)
CRRFQDC	(30)	1 (2.1%)
CLMGVHC	(31)	1 (2.1%)
CKLLSGVC	(32)	1 (2.1%)
CFVGHDLC	(33)	1 (2.1%)
CRCLNVC	(34)	1 (2.1%)
CKLMGEC	(35)	1 (2.1%)

* - numbers in parentheses indicate SEQ ID NO:.

searches did not reveal any significant homology of the
brain homing or kidney homing peptides to known ligands
for endothelial cell receptors.

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C. Peptide homing is specific:

In order to confirm the specificity of a
peptide for directing homing to a selected organ, peptide
competition experiments were performed. The cyclic
5   peptide, CLSSRLDAC (SEQ ID NO: 3), which is one of the
brain homing peptides (see Table 1) was synthesized
(Immunodynamics; La Jolla CA) and purified by HPLC. The
effect on the homing of phage expressing CLSSRLDAC (SEQ
ID NO: 3), CENWWGDVC (SEQ ID NO: 2) or
10   WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) to the brain was
examined in order to determine whether co-administration
of the synthetic peptide affected homing of the phage.

Phage expressing CLSSRLDAC (SEQ ID NO: 3),
CENWWGDVC (SEQ ID NO: 2) or WRCVLREGPAGGCAWFNRHRL (SEQ ID
15   NO: 16) were titrated to the same concentration and
1×10^8 TU was injected into mice alone, or with 100 μg
purified synthetic cyclic CLSSRLDAC (SEQ ID NO: 3)
peptide. The synthetic CLSSRLDAC (SEQ ID NO: 3) peptide
inhibited the homing of phage expressing CLSSRLDAC (SEQ
20   ID NO: 3) by about 60%. This result demonstrates that
the homing of the phage to brain is specifically due to
the expression on the phage of the CLSSRLDAC (SEQ ID
NO: 3) peptide.

The synthetic CLSSRLDAC (SEQ ID NO: 3) peptide
25   also inhibited homing of phage expressing the
WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) peptide by about
60%, but did not affect homing of CENWWGDVC (SEQ ID
NO: 2) phage. This result indicates that the CLSSRLDAC
(SEQ ID NO: 3) and WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16)
30   peptides can recognize the same target molecule in brain,
whereas the CENWWGDVC (SEQ ID NO: 2) peptide recognizes a
different target.

D. The brain homing peptide CLSSRLDAC (SEQ ID NO: 3) directs red blood cells to brain

The synthetic cyclic CLSSRLDAC (SEQ ID NO: 3)
5   peptide (1 mg) was labeled with iodine-125 using the
Bolton and Hunter reagent (Amersham; Arlington Heights
IL). Labeled peptide was purified by reverse phase HPLC
on Sep-Pak™ cartridges (Waters, Millipore Corp.; Milford
PA) and the ^{125}I-peptide (200 μg) was coupled to 1 ml
10 glutaraldehyde-stabilized sheep RBC (Sigma Chemical Co.;
St. Louis MO) according to the manufacturer's
instructions.

Fifty μl ^{125}I-peptide/RBC (200,000 cpm), alone
or with 10 mM of unlabeled CLSSRLDAC (SEQ ID NO: 3),
15 which is a brain homing peptide, or CVRLNSLAC (SEQ ID
NO: 43), which has no brain homing activity, was injected
into the tail vein. After 2 min, each mouse was perfused
through the heart with 50 ml DMEM and the brain and
kidney were removed and assayed for radioactivity.

20   Approximately twice as much of the
CLSSRLDAC/RBC complex (SEQ ID NO: 3) homed to brain than
to kidney. Coadministration of unlabeled CLSSRLDAC (SEQ
ID NO: 3) with the complex essentially completely
inhibited brain homing of the complex but had no effect
25 on complex localizing to the kidney, indicating that
localization of the complex in the kidney was
non-specific. Coadministration of unlabeled CVRLNSLAC
(SEQ ID NO: 43) had no effect on selective homing of the
complex to brain and no effect on the non-specific
30 localization of the complex in the kidney. These results
demonstrate that an organ homing peptide identified using
in vivo panning can be linked to a moiety such as a blood
cell and selectively directs homing of the linked moiety
to the selected organ.

E. *In vivo* panning for molecules that selectively home to an organ containing a component of the reticuloendothelial system

This example demonstrates that enhanced uptake
5   of peptide-displaying phage by the RES in liver and
spleen can be blocked by coadministration of
non-infective phage, thereby allowing identification of
molecules that selectively home to an RES-containing
organ.

10   Approximately 1 x 10^8 TU of amplified phage
expressing a CX_5 (SEQ ID NO: 39) library were injected,
iv, into mice, either alone or with a 1000-fold excess of
non-infective phage, which are not amplifiable. After
2 min, the mice were sacrificed and phage were rescued
15   from liver, lung, kidney, brain and spleen, and the
number of phage (TU) per gram (TU/g) of tissue recovered
was determined.

Coadministration of non-infective phage with
library phage substantially reduced the amount of phage
20   recovered in liver from about 1300 TU/g to about 200 TU/g
and, in spleen from about 300 TU/g to about 200 TU/g. In
comparison, little or no difference was observed in the
number of phage recovered from brain, kidney or lung when
the phage library was administered alone or in
25   combination with the non-infective phage. This result
demonstrates that nonspecific uptake of phage by organs
such as liver and spleen, which contain an RES component,
can be blocked by coadministration of non-infective phage
with a phage library.

30   These results also demonstrated that uptake of
phage by the lung was selective. Specifically, recovery
of phage from lung essentially identical regardless of
whether the phage library was injected alone or was

coadministered with non-infective phage. Thus, the high
uptake of phage previously observed in the lung
(Pasqualini and Ruoslahti, *supra*, 1996) likely is due to
the large amount of vasculature in the lungs and is not,
5 for example, due to uptake of the phage by alveolar
phagocytes, which constitute an RES component in lung.

In order to demonstrate that the phage that
homed to liver when coadministered with non-infective
phage, in fact, selectively homed to liver, a phage
10 library was coadministered with non-infective phage, then
phage recovered from the liver after a first round of *in
vivo* panning were amplified *in vitro* and used for a
second round of *in vivo* panning. Phage were recovered
from liver and from brain (control organ) and quantitated
15 after each round of *in vivo* panning.

Following the second round of *in vivo* panning,
more phage homed to liver (350 TU/g) as compared to brain
(200 TU/g). In addition, about 150 TU phage/g liver were
recovered after the first round, whereas about 350 TU
20 phage/g liver were recovered after the second round,
indicating that the population of phage recovered from
the liver following the first round of *in vivo* panning
was enriched with phage that selectively home to liver.
These results demonstrate that the RES component of an
25 organ such as liver can be blocked, thus allowing use of
the *in vivo* panning method to identify a molecule that
selectively homes to such an organ.

EXAMPLE III

CHARACTERIZATION OF PEPTIDES THAT HOME
TO A SELECTED TUMOR TISSUE

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This example demonstrates that *in vivo* panning
can be used to identify peptides that home to a breast
tumor or to a melanoma.

A. Peptides that home to a human breast carcinoma tumor implanted into a nude mouse

Human 435 breast carcinoma cells (Price et al., Cancer Res. 50:717-721 (1990)) were inoculated into the
5 mammary fat pad of nude mice. When the tumors attained a diameter of about 1 cm, either a phage targeting experiment was performed, in which phage expressing a specific peptide were administered to the tumor bearing mouse, or in vivo panning was performed.

10   The breast tumor bearing mice were injected with 1 x 10^9 phage expressing a library of CX$_3$CX$_3$CX$_3$C (SEQ ID NO: 47) peptides, where X$_3$ indicates three groups of independently selected, random amino acids. The phage were allowed to circulate for 4 min, then the mice were
15 anesthetized, snap frozen in liquid nitrogen while under anesthesia, and the tumor was removed. Phage were isolated from the tumor and subjected to two additional rounds of in vivo panning.

Following the third round of panning, phage
20 were quantitated and the peptide sequences expressed by 14 different cloned phage were determined. The 14 cloned phage expressed 4 different peptides (Table 3). These results demonstrate that the in vivo panning method can identify molecules that selectively home to breast tumor
25 tissue.

B. Peptides that home to B16 mouse melanoma cells implanted into a mouse

The general applicability of the in vivo panning method to identify peptides that selectively home
30 to a tumor was examined by performing in vivo panning in mice bearing an implanted mouse melanoma tumor.

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TABLE 3

PEPTIDES FROM PHAGE RECOVERED FROM HUMAN BREAST CANCER

SEQUENCE		
$CX_3CX_3CX_3C$ (47)* library	# clones	% of total phage sequenced
CGRECPRLCQSSC (48)	6	42.9
CGEACGGQCALPC (49)	4	28.6
CNGRCVSGCAGRC (50)	3	21.4
CGLMCQGACFDVC (51)	1	7.1

* - numbers in parentheses indicate SEQ ID NO:.

Mice bearing a melanoma were produced by implantation of B16B15b mouse melanoma cells, which produce highly vascularized tumors. B16B15b mouse melanoma cells were injected subcutaneously into the mammary fat pad of nude mice (2 months old) and tumors were allowed to grow until the diameter was about 1 cm. *In vivo* panning was performed as disclosed above. Approximately 1 x 10^{12} transducing units of phage expressing the CX_5C (SEQ ID NO: 36), CX_6C (SEQ ID NO: 37) or CX_5C (SEQ ID NO: 38) library were injected, iv, and allowed to circulate for 4 min. Mice then were snap frozen in liquid nitrogen while under anesthesia, tumor tissue and brain (control organ) were removed, and phage were isolated as described above. Three rounds of *in vivo* panning were performed.

The amino acid sequences were determined for the inserts in 89 cloned phage recovered from the B16B15b tumors. The peptides expressed by these phage were represented by two predominant sequences, CLSGSLSC (SEQ

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ID NO: 55; 52% of the clones sequenced) and WGTGLC (SEQ
ID NO: 52; 25% of the clones; see Table 4). Reinfection
of phage expressing one of the selected peptides resulted
in approximately three-fold enrichment of phage homing to
5   the tumor relative to brain.

TABLE 4

10   PEPTIDES FROM PHAGE RECOVERED FROM MOUSE B16B15b MELANOMA

SEQUENCE

CX_5C (36)* library	# clones	% of total phage sequenced
WGTGLC (52)	22	25%
CSVANSC (53)	3	3%
CSSTMRC (54)	2	2%
CX_6C (37) library		
CLSGSLSC (55)	46	52%
CX_7C (38) library		
CIEGVLGGC (56)	2	2%

* - numbers in parentheses indicate SEQ ID NO:.

30   Localization of the phage expressing a tumor
homing peptide in the mouse organs was examined by
immunohistochemical staining of the tumor and various
other tissues. 1 x 10⁹ pfu of a control (insertless)
phage or a phage expressing the tumor homing peptide,
35   CLSGSLSC (SEQ ID NO: 50), were injected, iv, into tumor
bearing mice and allowed to circulate for 4 min. The
mouse then was perfused with DMEM and various organs,

including the tumor were removed and fixed in Bouin's
solution. Histologic sections were prepared and stained
using anti-M13 (phage) antibodies (see Pasqualini and
Ruoslahti, *supra*, 1996).

5   Strong immunostaining was evident in the
melanoma obtained from a mouse injected with phage
expressing the CLSGSLSC (SEQ ID NO: 50) tumor homing
peptide. Staining of the melanoma generally was
localized to the blood vessels within the tumor, although
10   some staining also was present in the tumor parenchyma.
Essentially no staining was observed in a tumor obtained
from a mouse injected with the insertless control phage
or in skin or in kidney samples obtained from mice
injected with either phage. However, immunostaining was
15   detected in the liver sinusoids and in spleen, indicating
that phage can be trapped nonspecifically in organs
containing RES.

        These results demonstrate that the *in vivo*
panning method is a generally applicable method for
20   screening a phage library to identify phage expressing
peptides that home to a selected organ or tissue,
including a tumor.

EXAMPLE IV

IN VIVO TARGETING OF A PHAGE EXPRESSING AN
25   AN RGD PEPTIDE TO A TUMOR

        Human 435 breast carcinoma cells were
inoculated into the mammary fat pad of nude mice. When
the tumors attained a diameter of about 1 cm, phage
expressing a specific RGD-containing peptide were
30   administered to the tumor bearing mouse. Similar results
to those discussed below also were obtained with nude
mice bearing tumors formed by implantation of human

melanoma C8161 cells or by implantation of mouse B16
melanoma cells.

1×10^9 phage expressing the RGD-containing
peptide, CDCRGDCFC (SEQ ID NO: 57; see, Koivunen et al.,

5   supra, 1995) or control (insertless) phage were injected
intravenously (iv) into the mice and allowed to circulate
for 4 min. The mice then were snap frozen while under
anesthesia, and various organs, including tumor, brain
and kidney, were removed and the phage present in the

10   organs was quantitated (see Pasqualini and Ruoslahti,
supra, 1996).

Approximately 2-3 times more phage expressing
the CDCRGDCFC (SEQ ID NO: 57) peptide were detected in
the breast tumor as compared to brain and kidney,

15   indicating the CDCRGDCFC (SEQ ID NO: 57; RGD phage)
peptide resulted in selective homing of the phage to the
breast tumor. In a parallel study, unselected phage,
which express various, diverse peptides, were injected
into tumor-bearing mice and various organs were examined

20   for the presence of phage. Far more phage were present
in kidney and, to a lesser extent, brain, as compared to
the tumor. Thus, the 80-fold more RGD-expressing phage
than unselected phage concentrated in the tumor. These
results indicate that phage expressing the RGD-containing

25   peptide home to a tumor, possibly due to the expression
of the $\alpha_v\beta_3$ integrin on blood vessels forming in the
tumor.

Specificity of the breast tumor homing peptide
was demonstrated by competition experiments, in which

30   coinjection of 500 µg free peptide, ACDCRGDCFCG (SEQ ID
NO: 58) with the phage expressing the tumor homing
peptide reduced the amount of phage in the tumor by about
tenfold, whereas coinjection with the inactive control
peptide, GRGESP (SEQ ID NO: 59) essentially had no

effect. Immunohistochemical staining for the phage that
homed to the breast tumors showed accumulation of the RGD
phage in the blood vessels within the tumor, whereas no
staining was observed in brain or kidney (control
5   organs). These results demonstrate that phage displaying
a peptide that can bind to an integrin expressed on
angiogenic vasculature can selectively home *in vivo* to an
organ or tissue such as a tumor containing such
vasculature.

10   Although the invention has been described with
reference to the disclosed examples, it should be
understood that various modifications can be made without
departing from the spirit of the invention. Accordingly,
the invention is limited only by the following claims.

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CLAIMS

We claim:

1. A method of obtaining a molecule that homes
to a selected organ or tissue, comprising the steps of:

5   a. administering to a subject a library
of diverse molecules;

          b. collecting a sample of the selected
organ or tissue; and

          c. identifying a molecule that homes to
10   said selected organ or tissue.

          2. The method of claim 1, wherein each of said
diverse molecules is linked to a tag.

          3. The method of claim 2, wherein said tag is
a support.

15   4. The method of claim 1, wherein said library
is a phage display library.

          5. The method of claim 4, wherein said
molecule is a peptide.

          6. The method of claim 1, wherein said library
20 is selected from the group consisting of a nucleic acid
library and a chemical library.

          7. The method of claim 1, wherein said
selected organ or tissue is selected from the group
consisting of brain and kidney.

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8. The method of claim 1, wherein said selected organ or tissue is a tumor.

9. A peptide that selectively homes to brain, said peptide having the amino acid sequence:

5   X_1SRX_2 (SEQ ID NO: 45),

wherein X_1 and X_2 each is about 1 to about 10 independently selected amino acids.

10. The peptide of claim 9, said peptide having the amino acid sequence CLSSRLDAC (SEQ ID NO: 3).

10   11. The peptide of claim 9, said peptide having an amino acid sequence selected from the group consisting of CNSRLHLRC (SEQ ID NO: 1) and CNSRLQLRC (SEQ ID NO: 5).

12. A peptide that selectively homes to brain,
15 said peptide having the amino acid sequence:

              X_3VLRX_4 (SEQ ID NO: 46),

wherein X_3 is absent or is about 1 to 10 independently selected amino acids and X_4 is about 1 to about 20 independently selected amino acids.

20   13. The peptide of claim 12, said peptide having an amino acid sequence selected from the group consisting of CVLRGGRC (SEQ ID NO: 4) and WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16).

14. A peptide that selectively homes to brain,
25 said peptide having an amino acid sequence selected from the group consisting of CENWWGDVC (SEQ ID NO: 2) and CGVRLGC (SEQ ID NO: 6).

15. A peptide that selectively homes to brain, said peptide having an amino acid sequence selected from the group consisting of CKDWGRIC (SEQ ID NO: 7); CLDWGRIC (SEQ ID NO: 8); and CTRITESC (SEQ ID NO: 9).

5   16. A peptide that selectively homes to kidney, said peptide having an amino acid sequence selected from the group consisting of CLPVASC (SEQ ID NO: 21); CGAREMC (SEQ ID NO: 22); and CKGRSSAC (SEQ ID NO: 23).

10   17. A peptide that selectively homes to kidney, said peptide having an amino acid sequence selected from the group consisting of CWARAQGC (SEQ ID NO: 24); CLGRSSVC (SEQ ID NO: 25); and CTSPGGSC (SEQ ID NO: 26).

15   18. A peptide that selectively homes to a breast tumor, said peptide having an amino acid sequence selected from the group consisting of CGRECPRLCQSSC (SEQ ID NO: 48), CGEACGGQCALPC (SEQ ID NO: 49) and CNGRCVSGCAGRC (SEQ ID NO: 50).

20   19. A peptide that selectively homes to a melanoma, said peptide having an amino acid sequence selected from the group consisting of WGTGLC (SEQ ID NO: 52) and CLSGSLSC (SEQ ID NO: 55).

20. A peptide that selectively homes to a
25   melanoma, said peptide having an amino acid sequence selected from the group consisting of CSVANSC (SEQ ID NO: 53), CSSTMRC (SEQ ID NO: 54) and CIEGVLGGC (SEQ ID NO: 56).

21. A molecule that homes to a selected organ or tissue, said molecule identified by *in vivo* panning, comprising the steps of:

      a. administering to a subject a library
5   of diverse molecules;

      b. collecting a sample of the selected organ or tissue; and

      c. identifying a molecule that homes to said selected organ or tissue.

10   22. The molecule of claim 21, wherein said selected organ or tissue is selected from the group consisting of brain, kidney, a tumor and angiogenic vasculature.

      23. The molecule of claim 22, wherein said
15 tumor is selected from the group consisting of a breast carcinoma and a melanoma.

      24. A method of directing a moiety to a selected organ or tissue, comprising the steps of:

      a. linking the moiety to the molecule of
20   claim 21 to form a complex comprising said moiety and said molecule; and

      b. contacting said complex with the selected organ or tissue, wherein said molecule of claim 21 selectively binds to the selected
25   organ or tissue, thereby directing said moiety to said selected organ or tissue.

      25. The method of claim 24, wherein said contacting step is performed *in vitro*.

26. The method of claim 24, wherein said contacting step is performed *in vivo*.

27. The method of claim 24, wherein said moiety comprises a detectable label.

5   28. The method of claim 24, wherein said moiety comprises a cytotoxic agent.

29. The method of claim 24, wherein said moiety comprises a chambered microdevice.

30. A method of identifying the presence of a
10 target molecule, which specifically binds a molecule that selectively homes to a selected organ or tissue, comprising the steps of:

a. obtaining a sample of an organ or tissue suspected of expressing the target
15   molecule;

b. contacting said sample with the molecule of claim 21 under conditions that allow specific binding of said molecule of claim 21 with said target molecule; and

20   c. detecting specific binding of said molecule of claim 21, said specific binding identifying the presence of the target molecule in said sample.

31. A method of substantially isolating a
25 target molecule, which specifically binds a molecule that selectively homes to a selected organ or tissue, comprising the steps of:

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      a.   obtaining a sample of the selected organ or tissue, which expresses the target molecule;

5   b.   contacting said sample with the molecule of claim 21 under conditions that allow specific binding of said molecule of claim 21 with said target molecule to form a complex; and

10   c.   substantially isolating said target molecule from said complex.

32.   A substantially isolated target molecule obtained by the method of claim 31.

33.   A method of targeting *in vivo* an organ or tissue containing angiogenic vasculature, comprising 15 contacting the organ or tissue with a molecule that specifically binds an α_v-containing integrin.

34.   The method of claim 33, wherein said organ or tissue is a tumor.

35.   The method of claim 33, said molecule 20 selected from the group of an RGD-containing peptide and an antibody that specifically binds an α_v-containing integrin.

36.   The method of claim 33, wherein said α_v-containing integrin is $\alpha_v\beta_3$ integrin.

25   37.   The method of claim 33, wherein said molecule is CDCRGDCFC (SEQ ID NO: 57).

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   G01N33/567   G01N33/50   C07K1/04   A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   G01N   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 639 584 (INTERPHARM LABORATORIES LTD.) 22 February 1995	1-3,6, 21, 24-27,30
A	see the whole document	4,5, 7-20,22, 23,28, 29,31,32

A	WO,A,92 00091 (BIOLIGAND, INC.) 9 January 1992 see the whole document	1-37

A	EP,A,0 135 277 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 27 March 1985 see the whole document	1-37

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
20 January 1997	04.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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